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Description of the Southern swamp leech *Philobdella floridana* (Verrill, 1874) (Annelida: Hirudinea) from the Outer Banks, North Carolina, USA, with a revision of the species

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Abstract

The jawed leech *Philobdella floridana* (Verrill, 1874) occurs widely in swamps of the southeastern United States. The discovery of a population of *P. floridana* in Lake Phelps, an isolated lake in the Albemarle Peninsula in the Outer Banks region of North Carolina, is by far the northernmost record for this species. Description of the Lake Phelps leech in this paper is the first comprehensive account of the internal anatomy of *P. floridana.* Comparable internal descriptions of *P. floridana* from other geographic regions are also presented for the first time. This new understanding of the internal features of this species is the basis of a taxonomic revision of *P. floridana*.

All specimens examined in this study possessed remarkably similar jaws and teeth within the range of 22–26 per jaw, regardless of the size or origin of the leech. All mature specimens possessed external copulatory slits and pores which uniquely characterise the genus *Philobdella*. This study shows for the first time that these external features are attributable to a complex internal network of gastropores emanating from the crop caeca of the genital segments. Nonetheless, the reproductive anatomy of this species is by far the most rudimentary of any Hirudinidae in North America.

A major finding of this study is that the Lake Phelps population differs significantly from other known *P. floridana* in one significant respect. Specifically, the gut has an intimate anatomical association with the nephridia, an enteronephric feature previously unknown within the Hirudinea. At least in this study, enteronephry may be confined to Lake Phelps, leaving open the possibility that this population of *P. floridana* may be endemic to this relict lake which has a history of endemicity.

Key words: Albemarle Peninsula, Lake Phelps, endemic, gastropore, enteronephry

Introduction

In an ongoing eco-systematic study of the Hirudinea in the Albemarle-Pamlico region of the Outer Banks of northeastern North Carolina, a population of an unusual jawed leech of the genus *Philobdella* was encountered in a remote lake on the Albemarle Peninsula (Fig. 1). This unexpected discovery extends the known range of this genus along the east coast more than 300 km to the north.

This peculiar leech was encountered along the shoreline of Lake Phelps, an isolated lake with unusual geochemical and biological features, including the absence of an outlet to nearby rivers (Anonymous 1979; Sawyer 2010; Owen 2016). Unlike the mud-bottomed, black-water lakes typical of the vast Albemarle swampland, including Lake Drummond in the Great Dismal Swamp (Speiran *et al.* 2015), Lake Phelps has a clear sandy bottom more in common with Lake Waccamaw of southeastern NC. This Carolina Bay lake is renown for having several endemic species, notably fish. One of these, namely the Banded Killifish *Fundulus waccamensis* Hubbs and Raney, 1946, is reputedly shared with Lake Phelps (Krabbenhoft *et al.* 2008; Stuber & Scanlan 2016). Intriguingly, Lake Waccamaw is also the nearest known locality from Lake Phelps for *Philobdella* (Sawyer & Shelley 1976).

External examination confidently places this leech in the genus *Philobdella*, easily recognized by the copulatory slits and pores unique to this genus (Fig. 4A). Furthermore, its teeth and jaws (Fig. 5A, B) would

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identify it as being *P. floridana* (Verrill, 1874), a species originally described from south Florida. However, internal examination of this leech revealed a unique gut-nephridial association (Figs. 6, 7, enteronephry) not previously known in *Philobdella*, nor elsewhere in the Hirudinea.

Unfortunately, the internal anatomy of *P. floridana* from the type locality in Lake Okeechobee, FL, remains unknown. The only available internal description of this species is a competent, but incomplete, account nearly fifty years ago from South Carolina (Richardson, 1972). In view of these deficiencies this paper presents the first comprehensive internal description of *P. floridana*, based on the Lake Phelps leeches. Furthermore, comparable descriptions are presented on *P. floridana* from other geographic regions. By way of summary, this study suggests that the Lake Phelps population of *P. floridana* may be morphologically unique with respect to the gut-nephridial association, and gives credence to possible endemicity.

A corollary of this revision of *P. floridana* is elucidation of the nature of the copulatory complex characteristic of the genus *Philobdella.* The Lake Phelps material was particularly fresh and well preserved, and thereby revealed for the first time that the external slits and pores in the genital segments are actually gastropores created by contiguity between the crop caeca and the ventral body wall.

Materials and methods

Reference material. The following specimens from North Carolina (Lake Phelps), South Carolina (Santee Swamp) and Georgia (Okefenokee Swamp) were examined in this taxonomic study. All reference material is retained under the respective deposition numbers in the collections of the Medical Leech Museum, Swansea, UK. In order to facilitate comprehension regarding geographic origin, the deposition number is typically prefixed with the state abbreviation, e.g. NC(Phg-1).

NORTH CAROLINA**.** Two mature specimens of *Philobdella* were collected under bark of submerged logs on 6 May 2016 at Pettigrew State Park, Washington County, NC (35.790038, -76.410943). These were relaxed by slowly adding alcohol until completely devoid of movement. They were then flooded with 5% formalin for several hours and subsequently stored in 5% formalin, in preference to 10% formalin (which makes the tissue unacceptably brittle for dissection) or 70% ethanol (which dissolves pigmentation). Reference numbers are NC(Phg-1) and NC(Phg-2).

A single small specimen (about 10 mm) collected in Lake Phelps in July 1975 was found in the NC Museum of Natural Sciences (NCSM 47412). Although clearly in the Hirudinidae in having five pairs of eyes in an arc, it lacked the copulatory complex characteristic of the genus *Philobdella*. Its confirmation as an immature *P. floridana* is discussed below.

SOUTH CAROLINA. A mature specimen of *P. floridana* from Santee Swamp in the coastal plain of South Carolina was in the author's collection. It was collected on 27 February 1975 in a ditch near the bridge at Highway 41 and Santee River, Berkeley County, SC (33.304600, -79.678664). This specimen SC(Psa-1) had been stored in 5% formalin for over forty years, but the internal tissue was suitable for dissection.

GEORGIA. Two specimens of *P. floridana* were collected on 14 and 20 July 2017 by Jenn Hogan of Okefenokee Adventures, at Chesser Prairie, Okefenokee Swamp, Ware County, GA (30.730888, -82.175146), A preliminary account of these leeches has been presented elsewhere (Hogan & Sawyer 2018). They were initially preserved in 70% isopropyl alcohol, before being transferred to 5% formalin. The external features, along with teeth and jaws, retained integrity, apart from being devoid of pigmentation. However, only the larger specimen GA(Pfl-2) was adequate for dissection. Reference numbers are GA (Pfl-1) and GA(Pfl-2).

Dissecting technique. Dissections were carried out under dechlorinated water on individual specimens secured by insect pins in a wax-bottomed dissecting tray using a Wild M7A stereo microscope with a Volpi Intralux 4000 light source, aided by a Lapsun Spot Point LED lamp. Photographic images were taken with digital cameras of increasing magnification: 1) Conrad Electrics, Mirazoon MZ902, DP-M14; 2) Yuanj MC500 eyepiece camera for stereo microscope; and 3) Bresser LCD 5 MP Microscope for prepared slides. Measurements were taken by Ocular Micrometer Model WF10X.

External features, particularly those of taxonomic importance, were recorded for each specimen in this study. In addition, the larger and better preserved specimen from each of the three geographic localities was examined internally following a mid-dorsal incision. As key anatomical features were revealed during dissection, particularly

of the digestive and reproductive systems, these were photographed at appropriate stages for future reference and tracing onto Bristol board.

The jaws and teeth were examined and photographed following a mid-ventral cut of the mouth region; the jaws were normally stained with Haematoxylin (Harris), and wherever possible a single jaw was mounted onto a glass slide for counting teeth in profile.

Terminology. The segmental nomenclature used throughout this study is that conventionally recognized for the Hirudinea, as follows (see Sawyer 1986: 54–66). The leech body is comprised of 34 segments, each assigned a Roman numeral (I–XXXIV). Each segment is defined by its respective ganglion. Six fused ganglia (I–VI) constitute the head and seven fused ganglia (XXVIII–XXXIV) constitute the tail (caudal sucker). The intervening 21 ganglia (VII–XXVII) constitute the ventral nerve cord, a string of independent ganglia which serve as useful segmental landmarks. In this paper the word "ganglion" is usually abbreviated, followed by the respective Roman numeral. For example, ganglion nineteen is written as "g. XIX".

In jawed or hirudinid leeches a mid-body segment is comprised typically of five external annuli, labeled from anterior to posterior as b1, b2, a2, b5 and b6 (This is derived from the "primitive" condition in which a leech segment is notionally tri-annulate, a1, a2 and a3; in hirudinid leeches the first and third notional annuli are further subdivided into b1 and b2; and b5 and b6, respectively.) The middle annulus, a2, is the center of the segment, and is defined externally as the sensilla-bearing annulus, and internally by location of the respective ganglion. Thus, XII b2/a2 refers to the furrow between the second and third annuli of segment twelve. This is location of the male gonopore in the species under study.

Taxonomy. Until recently, taxonomy of the genus *Philobdella* has been disordered, resulting in misleading and erroneous identifications of *P. gracilis* as living in South and North Carolina (e.g. Richardson 1972; Sawyer & Shelley 1976). In this context I concur with, and gratefully acknowledge, the much needed revision by Phillips & Siddall (2005) and Moser *et al.* (2011). As currently understood, this genus consists of two recognized species, *P. floridana* (Verrill, 1874) which has 20–26 monostichodont teeth on each jaw and currently recorded from south Florida to southeastern North Carolina ; and *P. gracilis* Moore, 1901, which has 35–45 partially distichodont teeth on each jaw and recorded from the Mississippi drainage system (Moore 1901; Klemm 1982; 1985; Phillips & Siddall 2005; Moser *et al.* 2011; Hogan & Sawyer 2018).

Results

Description of *P. floridana* from North Carolina (Lake Phelps)

A representative mature individual NC(Phg-2) from Lake Phelps is described in detail below. A second mature specimen NC(Phg-1) is compared briefly at the end of this description.

External features NC(Phg-2).

This slender specimen was fixed in 5% formalin in a relaxed, elongated position. In mid-body the sides were more or less parallel up to the clitellar region which gradually tapered to terminate in a narrow, rounded head not distinct from the body. The maximum body width was in the posterior half of the animal, at which point the body was ovoid in cross-section. Neither the head nor caudal sucker was a prominent feature. The caudal sucker was noticeably smaller than the posterior third of body. Dimensions: total length, 67.0 mm; maximum body width, 9.0 mm; diameter of caudal sucker, 4.5 mm; body width at male opening, 6.2 mm; distance from head to male opening, 16.0 mm.

Pigmentation. In life the dorsum and upper side of the caudal sucker had an overall dull olive-green camouflage color onto which were darkly pigmented longitudinal stripes. Upon preservation the greenish pigment faded perceptibly (Figs. 1A, 2B). The venter and anterior half of the underside of the caudal sucker were uniformly lighter (Fig. 3C), lacking the olive-green background color as well as black pigmentation, apart from rare random splotches.

The pigmentation pattern of the dorsum was characterised as follows (Fig. 2B): a distinctive light mid-dorsal stripe extended continuously from head to tail, and was lacking black pigmentation. On either side of this light stripe were three irregular black stripes running the full length of the body. The paramedial stripe was broadest, and separated from the intermediate black stripe by an indistinct lighter stripe. The intermediate was vaguely chain-like

and noticeably more intensely black than the paramedial stripe. The paramarginal stripe ran indistinctly along the margins and it, along with the background color, was sharply demarcated from the venter which was virtually devoid of pigmentation.

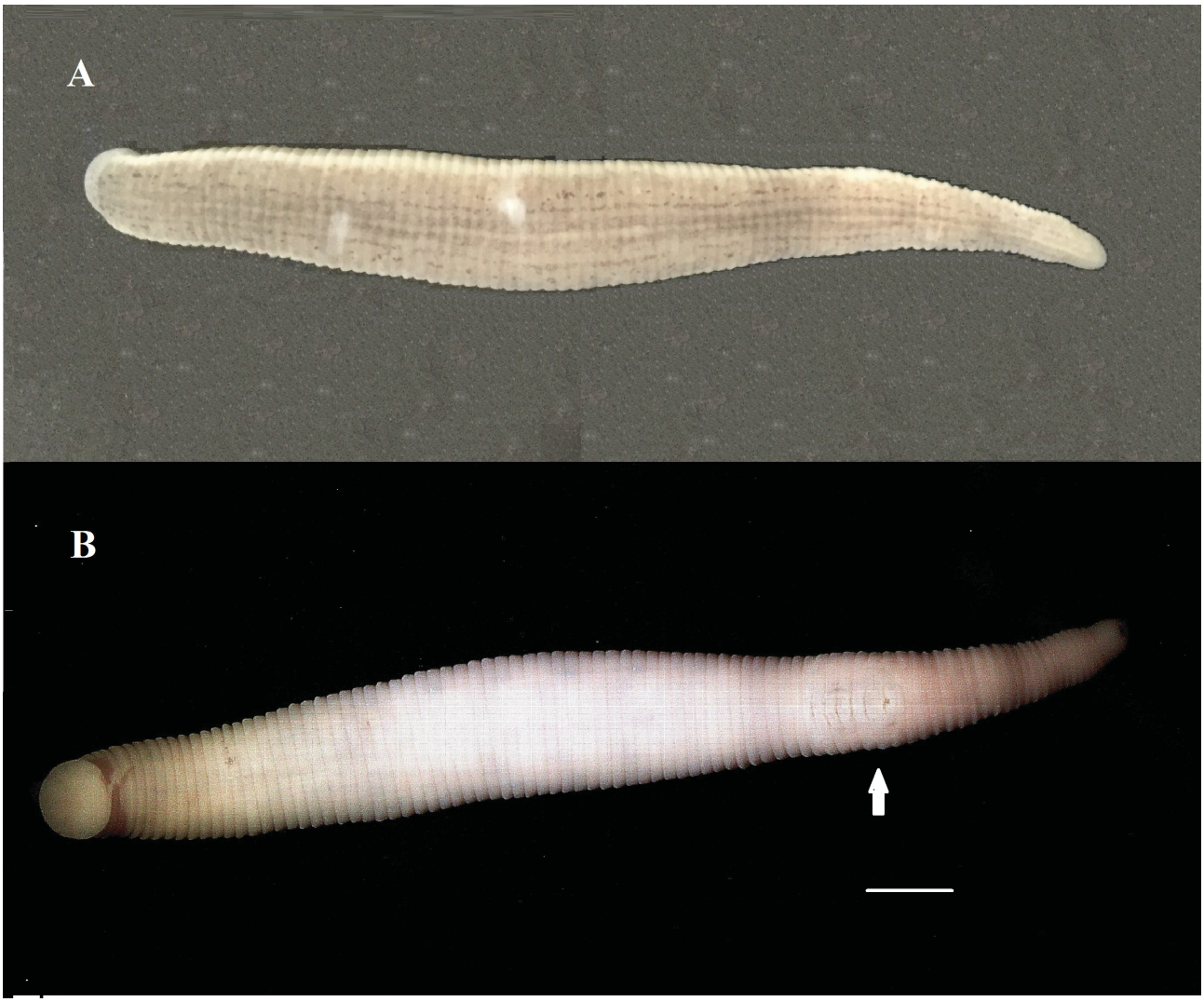

FIGURE 1. Newly discovered *Philobdella floridana* from Lake Phelps, Albemarle-Pamlico Peninsula of northeastern North Carolina (Phg-1). Anterior to right. A, dorsal view. B, ventral view of same specimen. Arrow: copulatory area typical of genus. Scale bar, 5.0 mm.

Nephridiopores. Seventeen pairs of ventral nephriopores occurred in segments VIII to XXIV, inclusively. Each was located in intermediate position at the posterior edge of the b2 annulus, almost into the furrow b2/a2. The nephridiopores varied in visibility, being more evident in mid-body and posterior segments, and least visible in anterior segments. In fact the nephriopores in segment VIII was barely, if at all, discernible in this specimen.

Sensilla. segmental sensilla were present on the dorsum on the a2 annulus. In this specimen these were small and relatively inconspicuous, often partially obscured by dark pigmentation. In a typical mid-body segment two pairs of dorsal sensilla were discernible in intermediate and paramarginal positions, respectively.

Eyes. Five pairs of eyes were arranged in a characteristic parabolic arc, a characteristic configuration for hirudinid leeches. The first pair of eyes was on segment II, second pair on III, third pair on anterior annulus of IV; fourth pair on anterior annulus of V; and the fifth pair was on middle annulus of VI.

Annulation. Annulation was reliably determined by invariant location of the Segmental sensilla and eyes in the notional a2 position, and nephridiopores in b2 position. Annulation on dorsum: Segment I, 1 annulus; II, 1; III, 1; IV, 2; V, 2; VI, 3; VII, 3; VIII, 4; IX–XXIII, 5; XXIV, 4; XXV, 4; XXVI, 2; XXVII, 1. Thus, this specimen unequivocally had 15 complete (i.e. 5-annulate) segments. This is a taxonomically important feature for American hirudinid leeches.

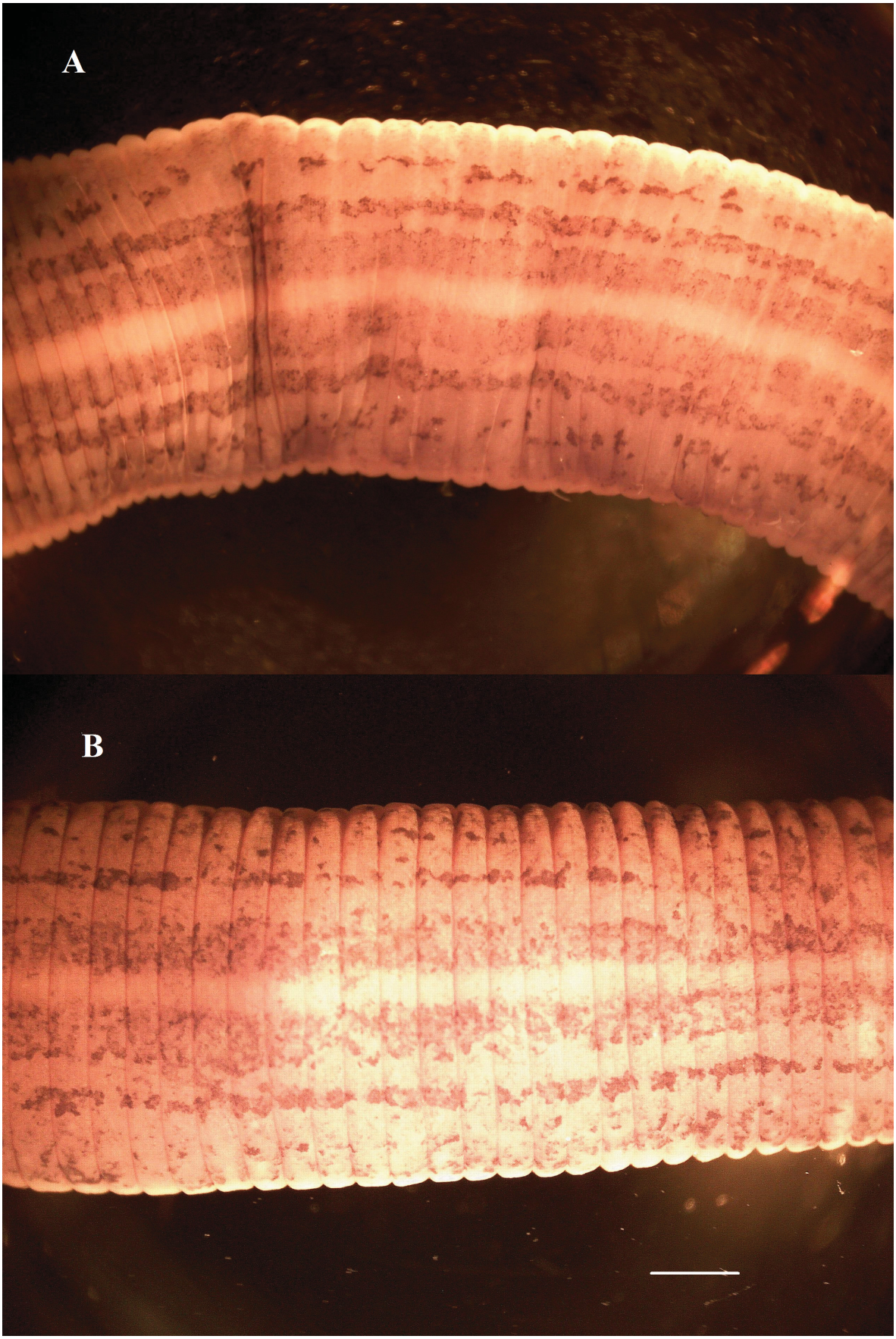

FIGURE 2. Similarity of dorsal pigmentation of *Philobdella floridana* from South Carolina and North Carolina. A, Santee Swamp, coastal SC (Psa-1). B, Lake Phelps, Albemarle Peninsula of northeastern NC (Phg-2). Scale bar, 2.0 mm.

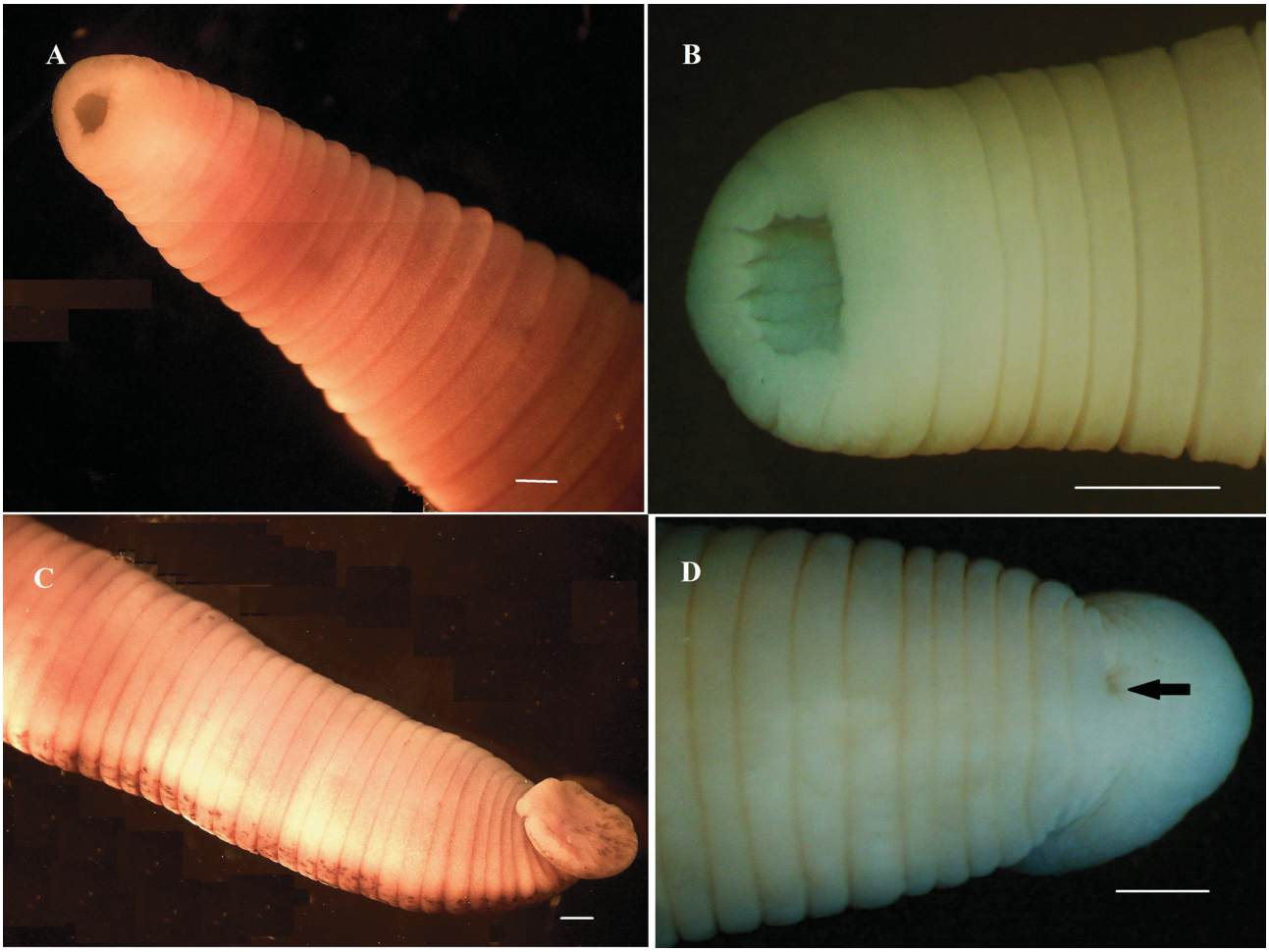

FIGURE 3. *Philobdella floridana* from Lake Phelps, NC (Phg-2) (A, C) and Okefenokee Swamp, GA (Pfl-2) (B, D). A, head and oral sucker, ventral view. B, head and oral sucker, ventral view. Note grooves leading into buccal region; C, caudal sucker, ventral view. Note partial pigmentation on under half of caudal sucker, venter of body unpigmented; D, caudal sucker with relatively large anus (arrow), dorsal view. All pigmentation in specimen from Okefenokee Swamp (B, D) bleached during prolonged exposure to isopropyl alcohol. Compare mouths of A, B with that of Fig. 4B in which the upper lip covers mouth. Scale bars, 1.0 mm.

Annulation of the head region was also taxonomically noteworthy, as follows: Segment IV had two annuli only evident dorsally (a1+a2, a3). Segment V had two annuli dorsally (a1+a2, a3) which fused ventrally (a1+a2+a3) to form the posterior rim of the oral sucker. Segment VI had three annuli dorsally (a1, a2, a3), the first two of which fused ventrally resulting in two annuli (a1+a2, a3) in this segment. Segment VII had three encircling annuli (a1, a2, a3) such that the a3 annulus was noticeably larger and tending to incipient subdivision dorsally, but not ventrally. Segment VIII had four encircling annuli (a1, a2, b5, b6), such that the first annulus (a1) was noticeably larger and tending to incipient subdivision dorsally, but not ventrally. Thus, the two adjacent annuli VII a3 and VIII a1 were enlarged and constituted a useful landmark evident both dorsally and ventrally.

Annulation of the caudal end was similarly foreshortened, but in a reversed manner compared to the head, as follows: Segment XXIV had four encircling annuli (b1, b2, a2, a3) such that the fourth annulus (XXIV a3) was noticeably enlarged dorsally and ventrally, and constituted a useful landmark. Segment XXV had basically four encircling annuli (b1, b2, a2, a3), but b1 and b2 are linked. Segment XXVI had two annuli (a1+a2, a3) evident dorsally but obscured ventrally by attachment to the sucker. Segment XXVII consisted of one annulus, behind which was the relatively small anus.

Oral sucker. The head narrowed dorsally into a rounded apex with no sucker nor neck discernible from above. Ventrally, the mouth was a spacious opening vaguely resembling a widely opened human mouth (Fig. 3A). This opening was widest at the lateral edges and was reminiscent of hinges supporting lower and upper lips. The slightly concave lower lip was formed by the large annulus V a1+a2+a3 and was edged with around six low fleshy

corrugations which descended into the buccal cavity. The upper lip was much more concave, and hinted at being bilaterally symmetrical, broadly curved at the apex. Each of its two sides was edged with roughly six low fleshly corrugations. From a distance the fully gaping mouth superficially appeared circular, but anatomically it constituted a broad triangle with the lower lip serving as its base. Anatomically the upper lip was supported by the first four segments, I–IV, which together appeared to be somewhat prehensile. The fulcrum was located between segments four and five, i.e. at IV a3/V a1+a2.

Caudal sucker. The caudal sucker was relatively small for the size of this individual and had a diameter less than the posterior third of the body (Fig. 3C). It was more or less circular with its attachment to the body appearing to be slightly anterior to center. The anus terminated on top of the sucker, there being no discernible post-anal annulus. The exposed dorsal portion of the sucker bore dark splotches. Ventrally, only the posterior portion of the sucker was pigmented.

Genital region. A distinctive clitellum indicated that this individual was sexually mature (Fig. 4A). This was manifested dorsally and especially ventrally as a lighter discolored area covering more or less fifteen annuli. It started precisely in the posterior half of annulus XI b1 (i.e. XI c2) and terminated at or just behind annulus XIII b5. Internally the clitellum consisted of a homogeneous layer of small, whitish cells of uniform size.

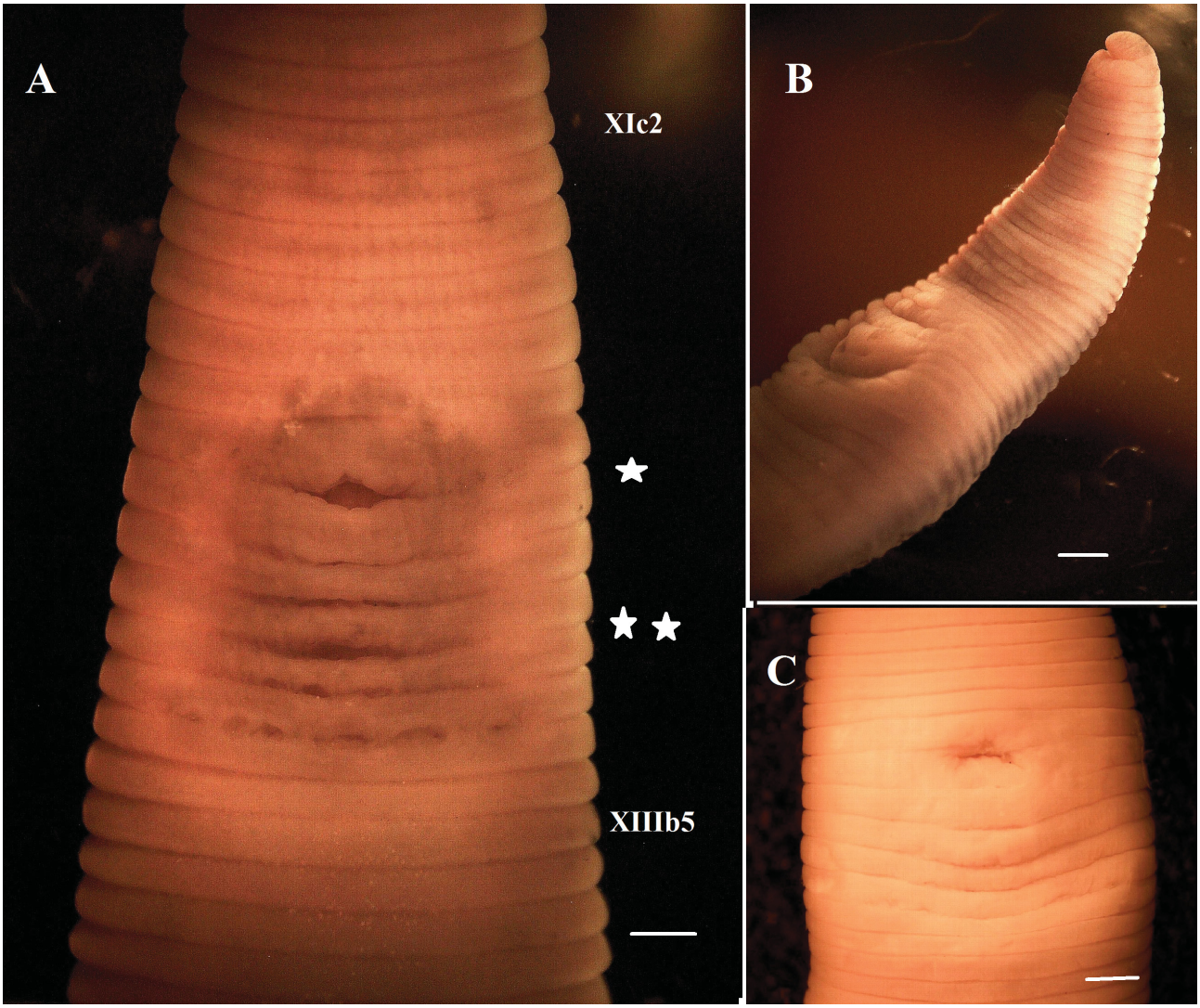

FIGURE 4. Copulatory complex of *Philobdella floridana* from North Carolina, South Carolina and Georgia. A, Lake Phelps, NC (Phg-2). Note distinct clitellum from annulus XIc2 to annulus XIIIb5, as indicated. B, Santee Swamp, SC (Psa-1). Specimen slightly distorted during preservation, showing deep saddle-shaped depression. Note prehensile upper 'lip' covering mouth. C, Okefenokee Swamp, GA (Pfl-2). Single star, male gonopore; double star, female gonopore, three annuli posterior to male. Scale bars, 1.0 mm.

On the ventral surface of segments XII and XIII was a discoloured copulatory region unique to the genus *Philobdella*. This area was a complex of conspicuous transverse slits confined to seven adjacent annular furrows. At the bottom of some of these furrows were a few small pore-like openings. Internally, these inconspicuous recesses appeared to be bilaterally symmetrical gastropores in that they were manifestations of the contact to the ventral body wall of the crop caeca of segments XII and XIII, respectively. These peculiar, previously unknown specializations of the caeca in the two genital segments of *Philobdella* are described below in the context of the gut.

Externally, the largest feature of the ventral copulatory complex was a deep transverse invagination of the tegument at the midline of XII b2/a2 (Fig. 4A, single star). This invagination was bordered by two swollen, lip-like annuli (XII b2 and XII a2, respectively). This depression sloped somewhat anteriorly where it shortly met a wide-mouthed pouch. The latter was a short penis-like bursa revealed in dissection. At the lateral edges of this male opening, just medial to the respective fifth pair of nephridiopores (NP5), was a short, shallow longitudinal depression which bore an inconspicuous gastropore. The latter was determined internally as arising from the crop caeca of segment XI. One annulus anterior to the male opening, in the furrow XII b1/b2, was a short transverse, discolored slit.

Three annuli behind the male opening was the female counterpart, a deep furrow located at the midline of XII b6/ XIII b1 [Inexplicably in this individual, at the bottom of this furrow there were actually two closely positioned openings, one larger than the other, rather than one].

Apart from the two annular slits associated with the male opening (i.e. furrows XII b1/b2 and XII b2/a2) , the remaining five slits and their respective gastropores were centred around the female opening. This is evident only from internal dissection. Thus, the two respective furrows anterior to the female opening, and likewise the two furrows posterior to the female opening, constituted an anterior-posterior annular symmetry around the female opening. It was not discernable externally, but internally this symmetry was attributable to the anatomical configuration of the gastropores (specialized crop caeca) of segment XIII. The first two aforementioned female-oriented furrows (i.e. XI Ia2/b5 and XII b5/b6) each displayed a gastropore on either side of, and well lateral to, the ventral midline. The last furrow (i.e. XIII b2/a2) has the widest and most specialized slit, which displayed at least two gastropores on each side of the ventral midline.

Internal features NC(Phg-2)

Digestive tract. **Foregut**. The buccal cavity was commodious and had a noticeably smooth lining with no apparent sulcus. In certain translucent light the more pronounced dorsal portion of this cavity extended posteriorly as far as segment VI. In this specimen the jaws were not visible through the mouth opening in that they were visually shielded by a tripartite velum, the base of which lay in ventral position. In dissection a slight cut along the mid-ventral line revealed three jaws (Fig. 5A), one in mid-dorsal position and a symmetrical pair in ventro-lateral positions. There was no apparent crypt for housing each jaw.

At its base each jaw was as wide as it was long, with a height about 70% of this dimension. In frontal view the jaw was roughly shaped like an isosceles triangle with its apex rounded rather than acutely pointed. In profile each jaw was broadly curved and bore distinctive individual teeth, contrasting white in relief in certain light (Fig. 5B). They were roughly of uniform height, i.e. straight rods without recurving tips (as in *Diplobdella* in Moore 1901b, fig. 13), and were arranged equidistant from each other. The right jaw had precisely 26 teeth, and the left jaw had at least 22 teeth. Those on the mid-dorsal jaw were present but barely visible due to the orientation of this dissection. The teeth appeared to be monostichodont with no evidence of distichodonty, at least in profile.

A well-developed pharyngeal ridge terminated between each jaw, i.e typical configuration for Macrobdellinae (Sawyer 1986: 461). In this specimen the distance between this ridge and the jaw was relatively spacious, suggesting the lumen could be expanded during ingestion. These ridges and those which form the jaws resulted from anastomoses of six to nine ridges which continued into the lumen of the pharynx. The encompassing circumpharyngeal commissure of the brain was the posterior limit of the jaw complex (Fig. 5A, b).

A prominent feature of each jaw was a pair of large bundles of salivary ductules which lay outside the circumpharyngeal commissure (Fig. 5A, s). The two bundles entered at the base of the jaw on each side. Each bundle coalesced with its partner about the middle of the jaw before splaying uniformly toward the rim. There was no clear indication of salivary papillae on sides of the jaws. Compared to most bloodsucking hirudinids, the jaws of this individual appeared to be poorly muscularized.

The salivary cells were remarkably prominent in the anterior segments, and aligned into six substantial bundles, two of which entered each jaw. The individual salivary cells extended posteriorly in the tissue nearly as far as g. XI. The salivary cells appeared to be independent throughout and, accordingly, did not constitute a true gland. These cells were starkly white and much larger in size than the aforementioned clitellar cells, or the voluminous 'botryoidal' tissue which abounded especially in the post-genital segments.

The pharynx was an elongated tube, neither robust nor bulbous, which extended posteriorly to g. IX (Fig. 6, p). The diameter of the circumpharyngeal commissure was only slightly smaller than that of the pharynx itself.

Midgut. The midgut was a flattened tube which extended from just behind g. IX, where it was not much wider than the pharynx at this point, to behind g. XIX. Its lumen was smooth walled in the anterior segments, but latterly bore some internal rugae. These become progressively developed toward the posterior portion of the midgut.

Throughout, the crop was unequivocally dichotomous, i.e. displaying two distinct pairs of unlobed caeca per segment (Fig. 6). In this individual these caeca were of unequal size with the anterior typically larger and more specialized than the posterior. To avoid confusion with respect to segmental landmarks, the posterior caecum lay over the respective ganglion (schematized in Fig. 7); furthermore, in the midgut each pair of caeca within a segment were delineated by an anterior and a posterior inter-segmental sphincter which protruded into the gut lumen, as described elsewhere.

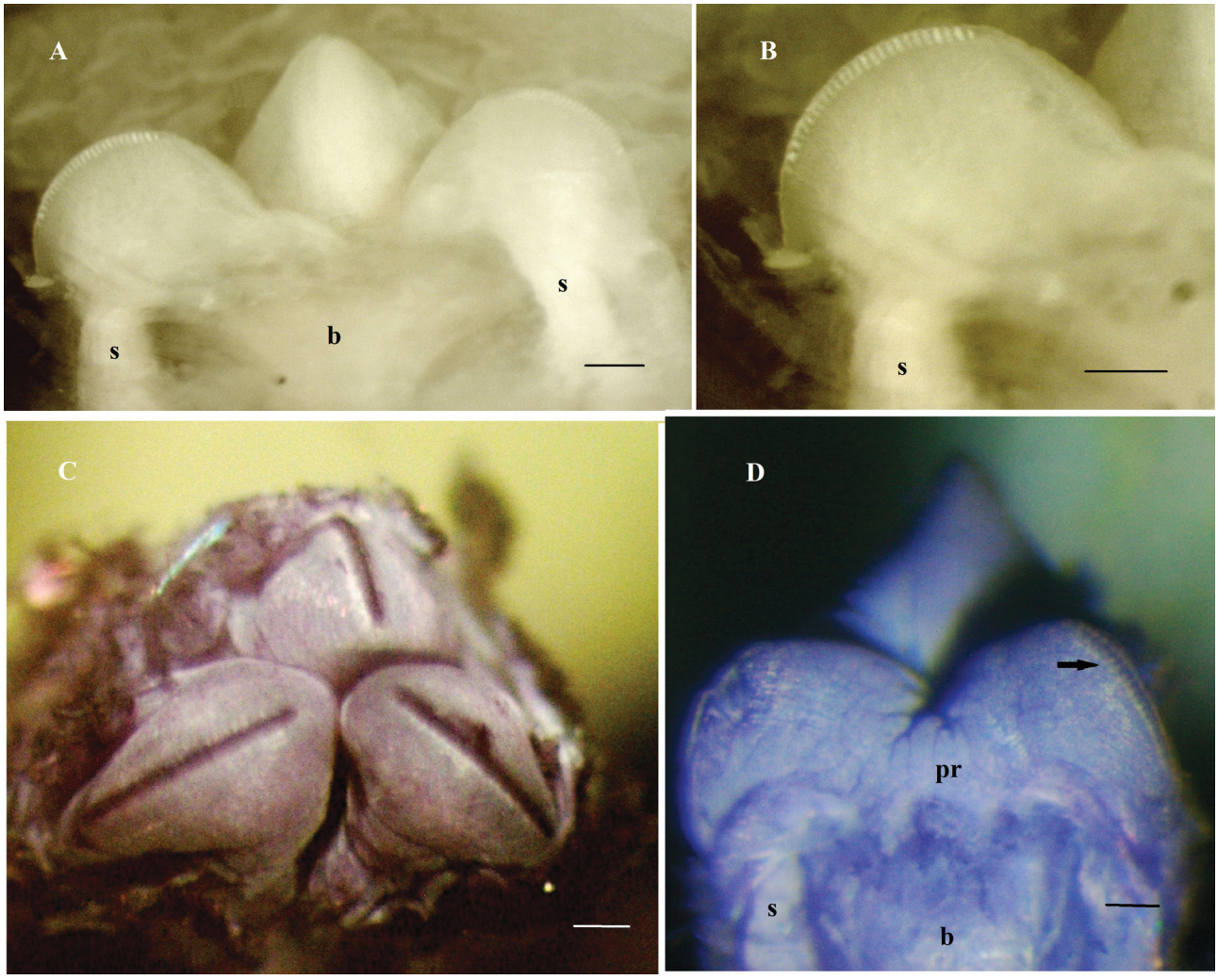

FIGURE 5. Jaws and teeth of *Philobdella floridana* from North Carolina (Lake Phelps, Phg-2) (A, B), South Carolina (Santee Swamp, Psa-1) (C) and Georgia (Okefenokee Swamp, Pfl-2) (D). Unstained (A, B); stained with Haematoxylin (Harris) (C, D). A, ventral view of jaw complex with mid-dorsal jaw in middle. Note the large salivary bundles entering base of jaws. B, profile of right ventro-lateral jaw showing 26 monostichodont teeth. C, frontal view of jaw complex showing location of teeth on rims. Note absence of salivary papillae on sides of the jaws. D, ventral view of jaw complex with mid-dorsal jaw in middle. Arrow indicates teeth on left ventro-lateral jaw. Note pharyngeal ridge terminating between two jaws, characteristic of subfamily Macrobdellinae. b, subesophageal ganglion or "brain"; s, salivary bundle; pr, pharyngeal ridge. Scale bars, 0.1 mm.

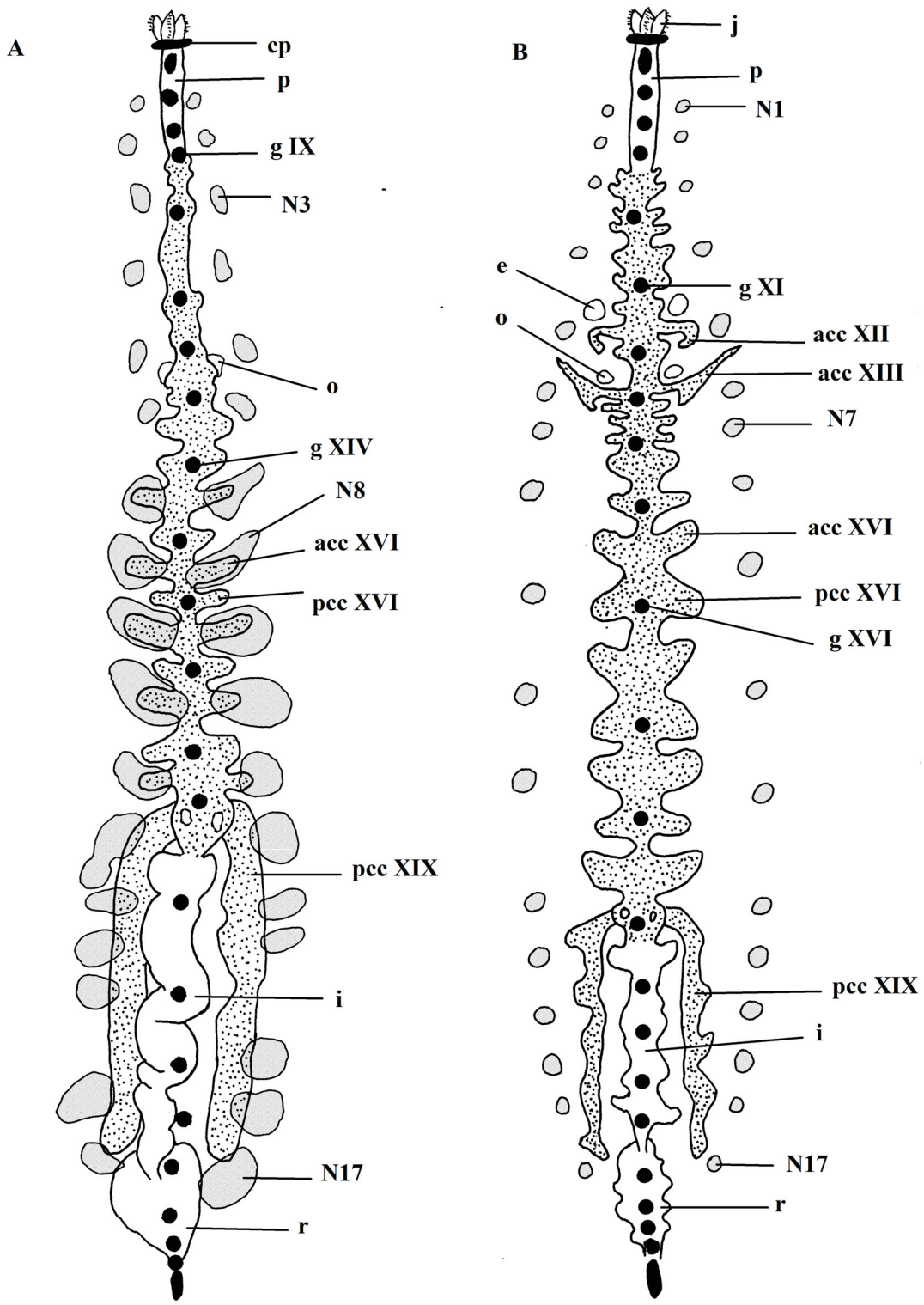

FIGURE 6. Comparison of digestive tracts of *Philobdella floridana* from North Carolina (Lake Phelps) (A) and South Carolina (Santee Swamp) (B), and their respective relationships to their urinary bladders. A, Dissected specimen Phg-2 showing prominent urinary bladders adhering to the anterior crop caeca of segments XV–XIX, respectively, and to the elongated posterior caeca of segment XIX (enteronephric). B, Dissected specimen Psa-1 showing that the 17 pairs of urinary bladders are physically independent of all crop caeca (i.e. not enteronephric). Note in both specimens the crops are dichotomous, i.e. an anterior and a posterior pair of caeca per segment. In specimen B the anterior caeca of segments XII and XIII are depicted descending to the ventral body floor; similar descension of these respective caeca also occurred in specimen A but is not depicted. Solid circles, segmental ganglia of ventral nerve cord, depicting 'brain' I–VI: ventral chain, VII–XXVII; tail mass, XXVIII–XXXIV.

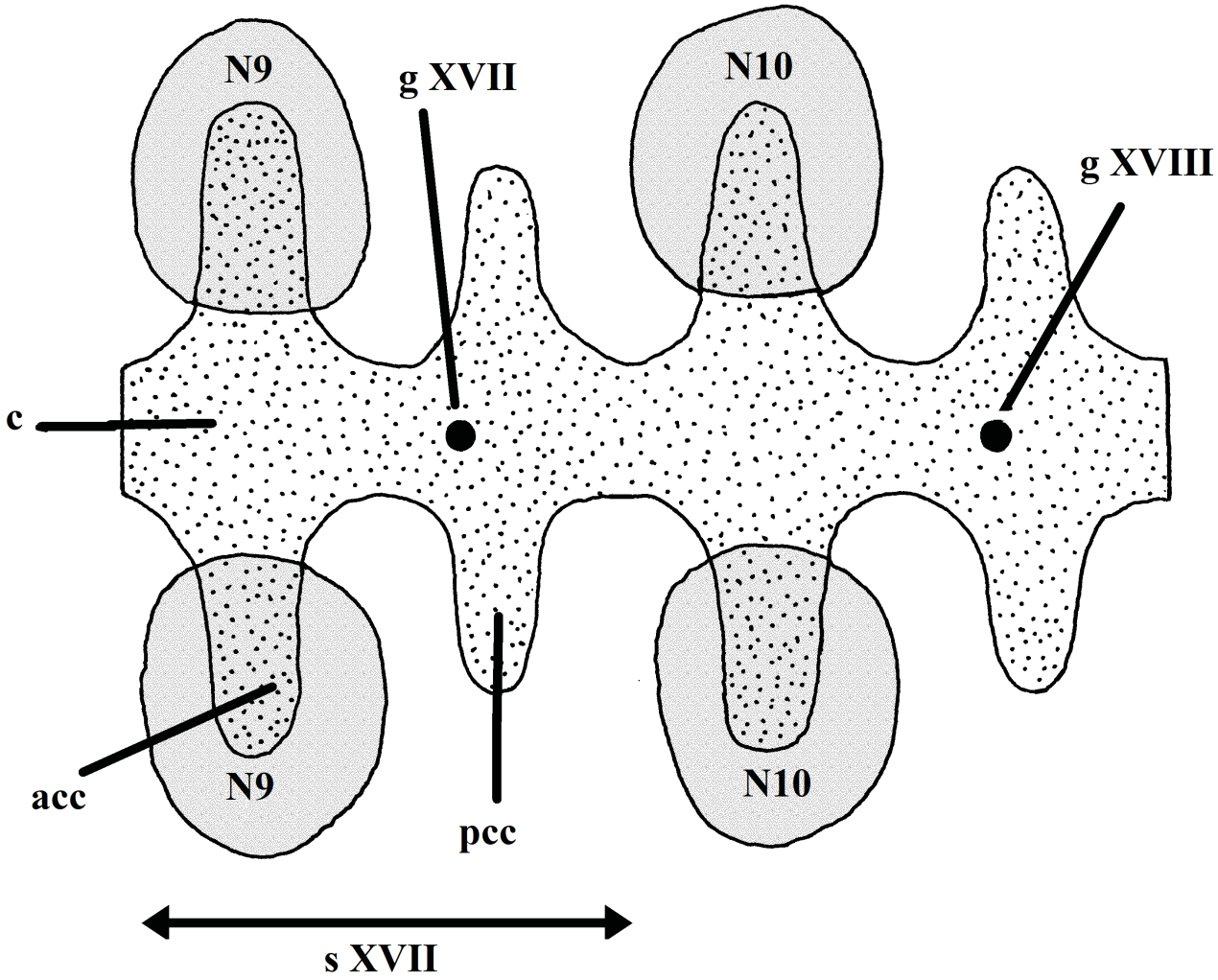

FIGURE 7. Stylized scheme depicting enteronephry of *Philobdella floridana* from North Carolina (Lake Phelps), based on dissection of specimens NC(Phg-1) and NC(Phg-2). Shown are mid-body segments XVII and XVIII, highlighting the 9th and 10th pair of urinary bladders (N9 and N10) and their relationship to the respective crop caeca. Note the tight attachment of the urinary bladders to the anterior caeca, but not to the posterior caeca, of each respective segment. Line with arrows defines the longitudinal extent of a single segment (XVII), showing its dichotomous structure, i.e. comprised of a larger anterior caecum and a smaller posterior caecum in each segment. Note relative position of the ganglion in each segment. Anterior is left.

There was an anterior-posterior transition in size and development of the crop caeca. In the pre-genital segments IX–XI, the crop was relatively narrow with simple segmental protuberances. In segments XII and XIII the anterior, but not the posterior, caeca were highly specialized in that they deflected ventrally toward the ventral body wall, in association with the male and female structures, respectively (see Gastropores) (Figs. 8, 9, acc).

The configuration of the crop into larger anterior and smaller posterior caeca per segment was most developed in segments XIV to XIX, inclusively. In this midgut region the spacious lumen of the crop was compartmentalized by prominent segmental constrictions or gastric sphincters. The last gastric compartment, in segment XIX, was further subdivided into distinct sub-compartments by a secondary sphincter. The anterior sub-compartment of XIX was unremarkable, whereas the posterior had many rugae, and gave rise ventro-laterally to a very elongated caecum on each side (termed the "post caecum" in other leeches). This extended posteriorly on either side of the intestine as far as XXIV.

The posterior caeca in segments XIV through XVIII, inclusively, are unremarkable and more or less uniform. In contrast, the anterior caeca in segments XV through XIX adhered tightly on either side to immense, very prominent urinary bladders N7-N11 (see Nephridia) (Figs. 6A, 7). Furthermore, the elongated posterior caecum

originating in XIX adhered tightly to six mid-sized urinary bladders N12–N17. Interestingly, the tip of the post caecum adhered to the outer surface of the rectum on each side, as well as to the last urinary bladder N17. The tight confluence of the rectum, posterior crop caecum and the urinary bladder could be teased apart from one another.

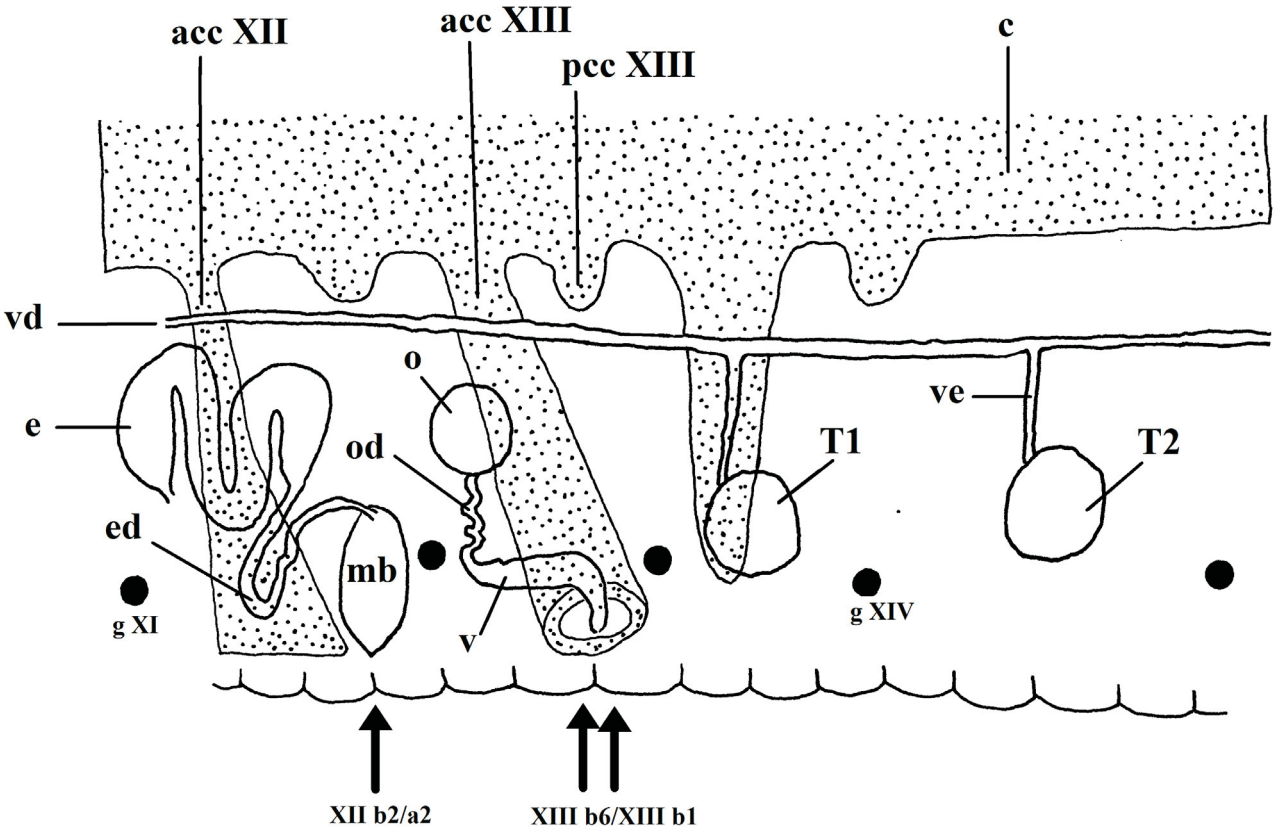

FIGURE 8. Lateral view of male and female reproductive structures of *Philobdella floridana* from North Carolina (Lake Phelps, Phg-2), showing complex relationship with the anterior crop caeca which extend to the ventral body wall (gastropores) in segments XII and XIII, respectively. Anterior is left. Arrow, position of male gonopore; double arrow, position of female gonopore. Inexplicably, in this specimen the vas deferens is atrophied on both sides of segment XI; the epididymis is missing on the right side; the left oviduct enters the end of the vagina, there being no vaginal caecum. Note: In this specimen the anterior caeca of segment XIV associate with the first pair of testisacs, but do not extend to the ventral body wall in this segment.

Intestine and rectum. The intestine was a conspicuous, robust tube which originates at XIX/XX at a shunt-like sphincter with a narrow lumen. The intestine convoluted broadly two or three times along its length, and for the most part lacked segmental landmarks. The exception was a prominent wide anterior caecum just behind the sphincter at XIX/XX. The intestinal lumen was characterised by numerous rugae which greatly increased the surface area. These resembled microvilli but were configured as high parallel ridges rather than finger-like protuberances. Unlike the anterior caeca of the midgut segments the intestine was anatomically independent of urinary bladders. The intestine tapered to the level of g. XXIV where it entered the dorsal part of the rectum.

The rectum was a conspicuous large bladder-like sac which extended from XXIII/XXIV to the anus. It was noticeably darker than the adjoining intestine. The thin, but tough, wall of the rectum was encircled by a patchy unilayer of longitudinal muscle fibers. The wall was translucent enough that the particulate contents of the rectum were just visible. The inner surface was relatively smooth throughout, and essentially lacked rugae.

Gut contents. Revealingly, the digestive tract of this specimen contained several large oligochaetes or parts thereof. In addition, some dark amorphous material occurred sporadically within the crop, and was particularly noticeable in the caeca of segment XIV. This greyish spongy-like substance was interspersed with some smallish particulate matter of varying sizes and colors. Overall, this ingested material appeared to consist primarily of detritus, with remarkably little grit.

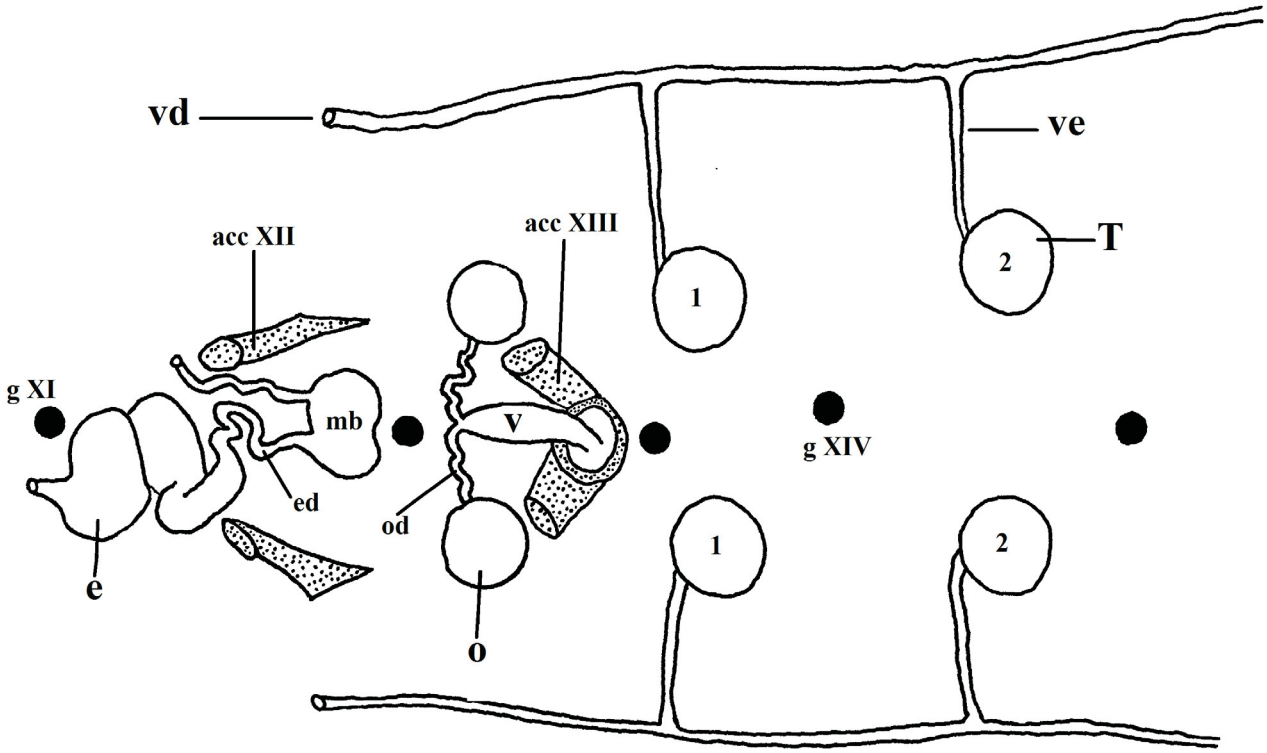

FIGURE 9. Dorsal view of the same specimen of *P. floridana* from North Carolina (Lake Phelps, Phg-2).

The gut contents were unevenly distributed. A recently ingested oligochaete was found in the anterior crop behind the pharynx (Fig. 13A), but most partially digested oligochaetes accumulated toward the posterior part of the midgut. Crop compartment XIX was gizzard-like and was particularly congested with partially digested oligochaete segments, along with some of the amorphous material mentioned above. The lengthy post caeca of XIX on either side of the intestine were replete with a dark compacted material which may have become hardened during preservation. The only clue as to its composition was a sizeable oligochaete which extended intact from the posterior sub-compartment XIX well into the right post caecum.

The intestine, unlike the midgut, was virtually empty apart from a clear, watery liquid. On the other hand the liquid content of the rectum was somewhat discolored by a fine suspension. Furthermore, the rectum contained clumps of amorphous material, interspersed within which were irregular lengths of what appeared to be undigested oligochaete cuticle.

Nephridia. Seventeen pairs of nephridia (N1–N17) lay on either side of the gut from segment VIII to XXIV, respectively (Fig. 6A). Each nephridium reaches the ventral body wall as an external nephridiopore (NP1–NP17) slightly anterior and lateral to the respective segmental ganglion. Each nephridium consisted of a nephridial canal anterior to which was a urinary bladder. In nephridia N2-N6 the urinary bladders were relatively small, roughly about the same size as the nephridial canal, and were independent of the gut in these anterior segments.

Inexplicably, N6 differed in that it was not in linear alignment with the other nephridia, being noticeably closer to the mid-line. In fact, its nephridial canal on each side lay medial to the vas deferens, whereas its urinary bladder lay lateral. In the other nephridia in this region both the nephridial canal and the urinary bladder lay in a position lateral to the vas deferens. It was probably significant that N6 was associated with the female segment XIII.

The remaining 11 nephridia N7–N17 were very peculiar in having intimate physical adherence to the gut (enteronephry) (Figs. 6A, 7). The urinary bladders were exceedingly large and adhered tightly to the respective segmental caeca of the midgut. Each contained a clear fluid, and the inner lining was smooth. Each of the five bladders N7–N11 was an elongated membranous sac which arose from the ventral floor and proceeded dorsally and posteriorly to adhere only to the larger (anterior) of the two crop caeca characteristic of each of segments XV–XIX, respectively. This adherence was tight and the bladder portion encompassed most of the respective caecum, like a finger in a water balloon. The remaining six bladders N12–N17 adhered to the very elongated last or post caecum

extending from segment XIX, and had no adherence to the intestine itself. The last urinary bladder N17 not only adhered to the tip of the post caecum, it also formed a close association with the rectum.

A second specimen NC(Phg-1) from the Lake Phelps population also prominently displayed the same enteronephry, i.e. a tight adherence of very large bladders to the gut, with some minor differences. The urinary bladders N12–N13 adhered not only to the post caeca extending from segment XIX, but also had broad contact with the intestine itself. In NC(Phg-2) the urinary bladders had no physical contact with the intestine. Another difference is that the urinary bladders in NC(Phg-1) did not encompass the crop caeca as entirely as in NC(Phg-2). Of taxonomic significance, this adherence of the gut with nephridial bladders is characteristic only of the population of *P. floridana* from Lake Phelps, and in this study was not found in this species from other geographic areas.

Reproductive structures NC(Phg-2)

Gastropores. In segments XII and XIII the crop caeca formed anatomical attachments to the ventral body wall (Figs. 8, 9) and were discernible externally as pores and slits in the vicinity of the male and female openings, respectively (Fig. 4A). These specializations derived only from the anterior caeca in these two respective segments, the corresponding posterior caeca being simply lobed and otherwise unremarkable. These presumptive gastropores constituted the structural basis of the peculiar copulatory region characteristic of the genus *Philobdella*. The presence of gastropores is unknown for any other leech endemic to North America (see Sawyer 1986: 510–514).
In segment XII the anterior caecum on either side descended from the ventro-lateral portion of the crop very close to the posterior end of the epididymis. It attached to the ventral body wall roughly lateral to halfway between g. XI and g. XII. This attachment splayed posteriorly as a hollow membrane as far as halfway between nephridiopore NP5 and the male opening, i.e. corresponding to the conspicuous external furrow XII b2/a2. The cavity within this caecum was continuous with the crop and extended intact to beyond the longitudinal muscle layer near the point of attachment to the ventral wall.

In segment XIII the anterior caecum was even more specialized and extensive than its male counterpart in segment XII. As viewed from above the lateral floor of the crop had three deep clefts, shaped like an upside down 'Y':

1) With respect to the anterior cleft its lateral edge formed a tight bond with the mesial half of the spheroidal ovisac, not unlike a hand holding a ball. The lateral half of the ovisac hemisphere was exposed and had no contact with the crop. This cleft was capacious and extended medially and anteriorly to the ventral wall to join its counterpart at the midline a short distance anterior to the female opening (XII b6/ XIII b1), at about the level of the distal end of the vagina. The cavity appeared to reach near or to the midline.

2) The mesial cleft was broad and descended ventrally where it attached to the ventral floor. This attachment splayed medially and posteriorly to form a hollow membrane or wall which terminated in the ventral floor very slightly anterior to g. XIII, a short distance behind the female opening. It met its bilateral counterpart under the nerve cord at the midline. Externally, this conjunction would be approximately at the furrow XIII b2/a2.

3) The lateral cleft descended behind the posterior face of the ovisac and was readily visible from outside the crop as a distinct descending arm of the caecum. This arm was also tightly contiguous with the body of nephridium N6, which was inexplicably out of linear alignment with the other nephridia of each side. This cleft reached the ventral wall where its attachment splayed posteriorly.

Taking the right and left sides together, these cavity-filled clefts from the anterior caecum of segment XIII reached the ventral floor in such a way that the female opening was encircled. They met their bilateral counterparts anterior and posterior to the female opening. The cavities were capacious and in each case extended continuously from the gut to below the longitudinal muscle layer of the ventral body wall. It could not be established whether the cavities conjoined with their bilateral counterparts at the midlines anterior and posterior to the female gonopore.

The external pits of the copulatory region were probably functional gastropores for the following reasons: 1) Internally, the lumen of the gut was continuous through the cavities of the respective caeca to beyond the longitudinal muscle layer at the ventral floor. 2) Externally, the pits were bilaterally symmetrical, lay at the bottom of the furrow slits, were circular in form, and the bottoms of the pits were visually unfathomable. 3) By gently squeezing the body of an intact specimen NC(Phg-1) some fluid *might* have been exuded via the pits. However, by way of caveat, whether the external pits in the copulatory region were continuously open to the gut lumen was not established by histology.

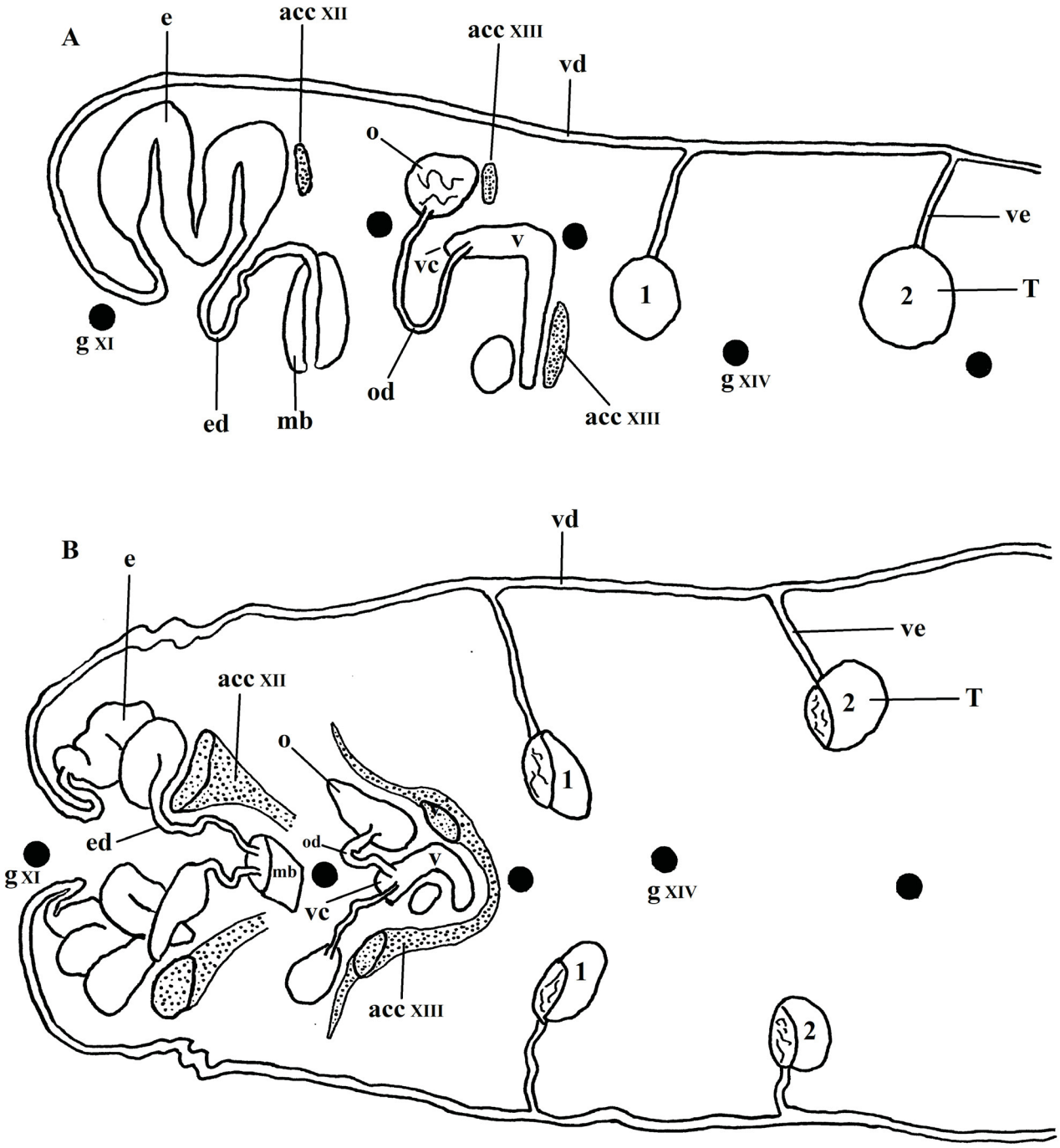

FIGURE 10. Male and female reproductive structures of *Philobdella floridana* from central South Carolina (Santee Swamp, Psa-1), showing relationship of the anterior crop caeca to the ventral body wall (gastropores) in segments XII and XIII, respectively. A, lateral view; B, dorsal view. Anterior is left. Note the oviducts enter the vagina subterminally, defining a vaginal caecum. The structure immediately anterior to the vagina remains unidentified.

Male system. Inexplicably in this specimen, certain paired seminal ducts were atrophied in segment XI. The proximal portions of the vas deferens were missing on both sides. Furthermore, the right epididymis was also missing, and its ejaculatory duct was underdeveloped. These aberrations evidently resulted from epigenetic factors within segment XI, and of no taxonomic significance.

The male median structure was micromorphic with little or no indication of a true morphological penis. Instead the male opening invaginated internally as a short, widened pouch or bursa just anterior to ganglion XII (Figs. 8, 9, mb). Emerging independently from the dorsal part of either side of this pouch was an ejaculatory duct. These were convoluted but not particularly well defined in this specimen.

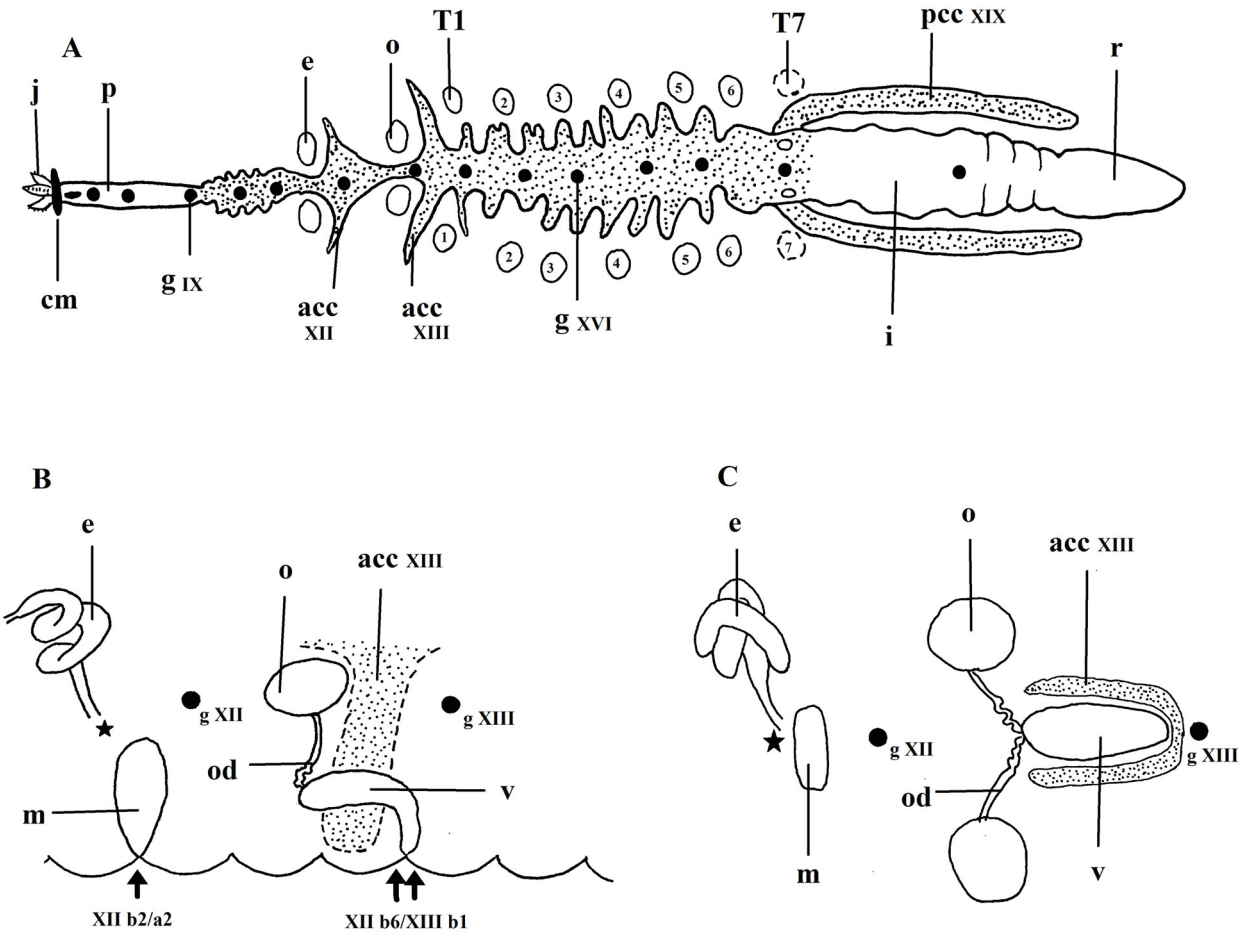

FIGURE 11. Digestive and reproductive systems of *Philobdella floridana* from Georgia (Okefenokee Swamp, based on dissection of specimen Pfl-2). A, Relationship of digestive system to the seven pairs of testisacs characteristic of this species. Note specialization of the anterior crop caeca of segments XII and XIII which deflect to the ventral body wall in these respective segments. B, C, Male and female structures. Anterior is left. B, lateral view. C, dorsal view. Note absence of a common oviduct, and lack of vaginal caecum. The ejaculatory ducts (star) probably connect independently to top of male bursa but tissue quality precluded certainty. Arrow, position of male gonopore; double arrow, position of female gonopore.

The left ejaculatory duct joined a conspicuous swollen epididymis which convoluted several times (Figs. 8, 9, ed, e). Its anterior tip, at about the level of g. XI, bore a very short duct which normally would have continued as the vas deferens. However, the proximal portions of both vasa deferentia in this region were missing altogether. They reappeared on each side at about the level of nephridium N5. They lay in the ventral floor just medial to the nephridia and proceeded posteriorly on each side with increasing integrity as far as XIX/XX.

Seven pairs of spherical testisacs lay anterior and lateral to g. XIV to g. XX, inclusively. The first testisac on each side was exceptional in adhering intimately to the anterior, but not posterior, crop caecum of segment XIV (Fig. 8, T1). In fact this caecum formed a deep pocket surrounding the anterior and lateral surfaces of this testisac. Unlike its segmental counterparts in segments XII and XIII, this caecum did not extend to the ventral floor.The next five pairs of testisacs lay beneath the larger, anterior caeca in each respective segment. The seventh pair lay beneath the posterior crop caeca on each side.

Each testisac was more or less spheroidal, and from its anterior ventro-lateral surface the vas efferens extended laterally to meet the vas deferens which proceeded anteriorly on each side along the ventral floor. The posterior portion of the vas deferens was large and convoluted, but became simpler as it proceeded anteriorly. Both the vas efferens and vas deferens contained a whitish flocculent material. Inexplicably, at about segment XI the vas deferens narrowed and emptied of content, making it difficult to discern further.

Female system. The vagina was unpaired and cylindrical in form (Figs. 8, 9, v). It originated at the ventral midline a short distance anterior to g. XIII. It arose vertically from the ventral floor a short distance before bending

anteriorly at right angles as a horizontal, elongated tube between the ventral floor and the crop. This distal portion of the vagina was longer than the short vertical section, and was more or less cylindrical throughout most of its length. Its distal tip ended at the level of the middle of the ovisacs.

The paired ovisacs were conspicuously large in this specimen, and was located lateral to, and just behind the level of, g. XII (Figs. 8, 9, o). Each ovisac was spheroidal and its contents were indistinctly visible through the translucent wall. From its ventro-medial surface was a convoluted oviduct which proceeded directly toward the midline to the vagina.

The two oviducts appeared to enter the vagina very close to, but apparently independently of, its bilateral counterpart, i.e. no common oviduct was evident (Fig. 8, od). Remarkably, they entered the vagina asymmetrically in that the left oviduct was continuous with the distal tip of the vagina, whereas the right oviduct entered ventral to, and was obscured by, the left oviduct. Because the left oviduct was continuous with the tip of the vagina, no vaginal caecum was possible. The asymmetry of the oviducts suggested that this configuration may be anomalous and of no taxonomic significance.

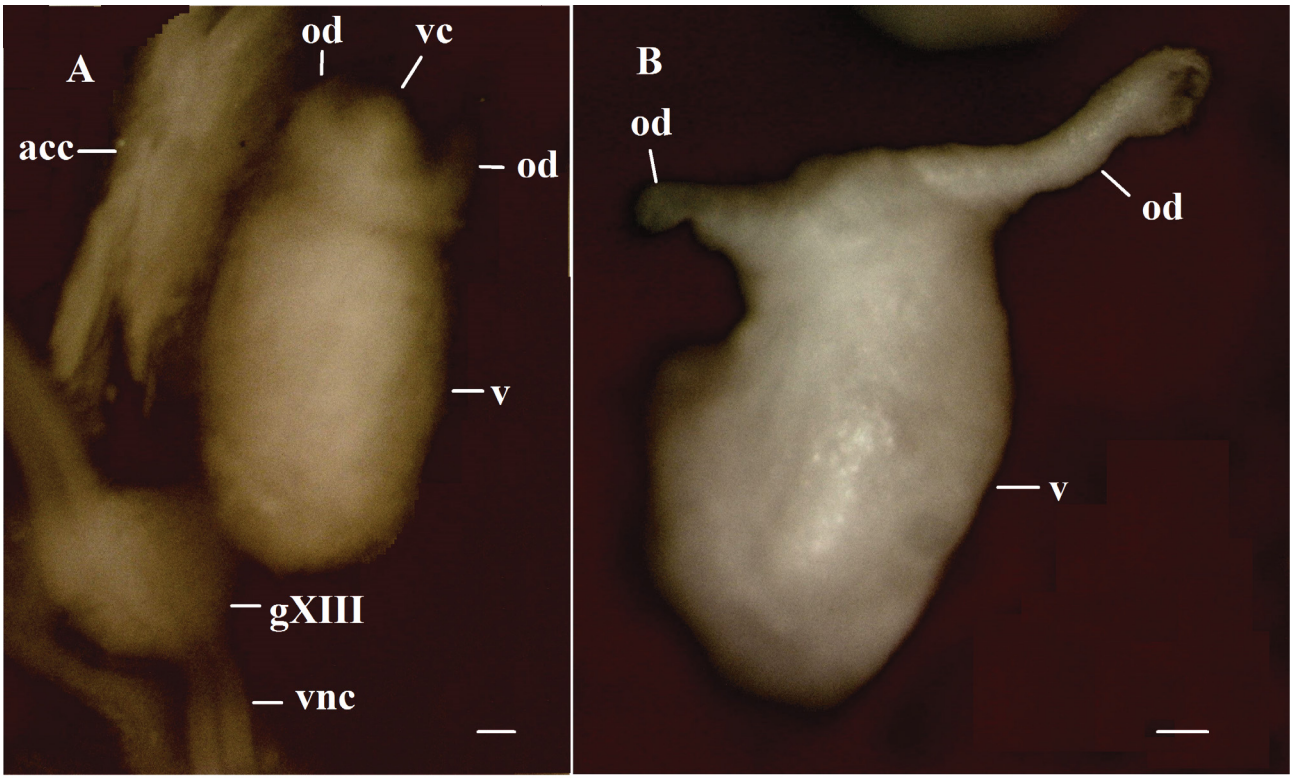

FIGURE 12. Comparison of the vaginas of *Philobdella floridana* from (A) South Carolina (Santee Swamp, Psa-1) and (B) Georgia (Okefenokee Swamp, Pfl-2). In both cases note absence of a common oviduct, a characteristic of the species. A, the two oviducts enter the vagina independently well behind its tip, thereby forming a distinct vaginal caecum. B, the two oviducts enter the vagina independently near its tip, such that there is no vaginal caecum. acc, anterior crop caecum of segment XIII descending to ventral body floor. Dorsal views; anterior is top. Scale bars, 0.1 mm.

Variation within the Lake Phelps population of *P. floridana*. Another mature specimen NC(Phg-1) was collected on the same day and circumstances as specimen NC(Phg-2) described in detail above. This slightly smaller (62 mm) individual was remarkably similar to NC(Phg-2) in nearly all external features, including a distinct clitellum, and a very similar copulatory complex (Fig. 1). The dorsum was dark with a mid-dorsal longitudinal stripe, and a sharp, lateral demarcation from the unpigmented venter. Segmental annulation was the same, including enlargement of adjacent annuli VII a3 and VIII a1 in the cephalic region, and annulus XXIV a3 in the caudal region. The presumptive male opening was also located within XII b2/a2. This specimen differed from NC(Phg-2) in that the three pairs of black longitudinal stripes were less defined, but the dorsal sensilla were more prominent. Segment XXV was 4-annulate in that annuli b1 and b2 were divided both dorsally and ventrally, slightly more distinctly than in NC(Phg-2). Internally, NC(Phg-1) also had intimate physical relationship between the nephridia and gut (enteronephry), the taxonomic significance of which is discussed elsewhere.

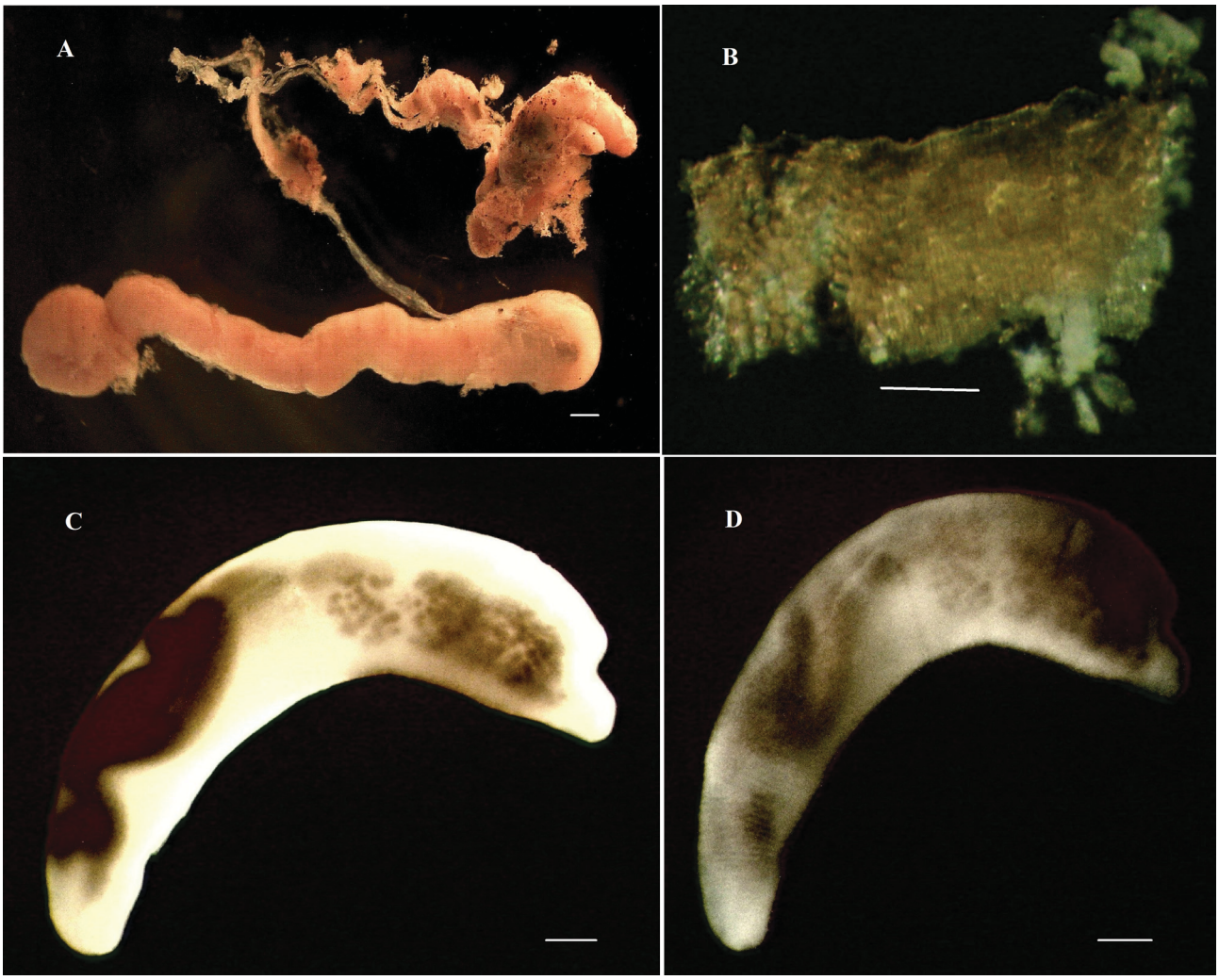

FIGURE 13. Representative gut contents of *Philobdella floridana* from Lake Phelps, NC (Phg-2) (A), Okefenokee Swamp, GA (Pfl-2) (B), and Santee Swamp, SC (Psa-1) (C, D). A, one of several oligochaetes found in the crop of this specimen. Note the two ends are connected by a thread of cuticle. B, one of several types of particulate matter found in crop, intestine and rectum, reminiscent of an undigested masticated exoskeleton. See also Hogan and Sawyer, 2018. C, D, single unidentified trematode found in upper intestine. C, transmitted light. D, reflected light. Scale bars, 0.2 mm.

An immature *P. floridana* (NCSM 47412) collected years earlier in Lake Phelps lacked the copulatory complex of adults. However, its gonopores were distinct dark slits located in furrows of indeterminate positions, and clearly separated by three complete annuli. The three-annulate separation of gonopores is a reliable diagnostic character for identifying immature individuals of this species. The late manifestation of gastropores in *P. floridana* implies that specialization of crop caeca in the genital segments XII and XIII probably develops as the animal matures.

This very small specimen (about 10 mm) resembled adult *P. floridana* from Lake Phelps in the following external characters: five pairs of eyes in a hirudinid arc, a mid-dorsal light stripe, and a sharp lateral demarcation between the dark dorsum and the unpigmented venter. Even in this small individual the adjacent annuli VII a3 and VIII a1 in the cephalic region were noticeably enlarged. Unfortunately, the teeth and jaws could not be accessed in this study.

Taxonomic comparison of the Lake Phelps population with *P. floridana* from other localities

The foregoing description of *P. floridana* from Lake Phelps is the first comprehensive account of the internal anatomy of this widely distributed, but poorly known, species. Thus, it is not known if the intimate relationship between the gut and nephridia (enteronephry) and, likewise, between the gut and reproductive system (gastropores) are restricted to the Lake Phelps population or are characteristics of all *P. floridana*.

Toward addressing these questions *P. floridana* from other parts of its range were dissected in comparable detail, with particular attention to the digestive, reproductive and nephridial systems. Selected for study were specimens from South Carolina and Georgia, representing distances from Lake Phelps of 400 km and nearly 800 km, respectively. Results are summarized graphically in Figs. 6B, 10, and 11.

Description of *P. floridana* from South Carolina (Santee Swamp)

A mature specimen SC(Psa–1) of *P. floridana* from Santee Swamp in the coastal plain of South Carolina was in the author's collection (see Reference material). The body of this individual was slightly flattened and tapered to a narrow head. Dimensions: length, 43 mm; maximum body width, 8.0 mm; diameter of caudal sucker, 3.5 mm. The relative width of the caudal sucker was smaller than in the Lake Phelps leech NC(Phg–2).

With respect to most external features, the Santee specimen was broadly similar to the Lake Phelps leech. This applied to dorsal pigment pattern (Fig. 2), number and location of eyes and nephridiopores. Common features of annulation included noticeable enlargement of adjacent annuli VIIa3 and VIIIa1. The posterior segments XXIV and XXV each had 4 annuli (b1, b2, a2, a3), respectively, making a total of fifteen 5–annulate segments IX–XXIII. Unlike the Lake Phelps leech (Fig. 3A) the upper lip of the Santee leech was folded over the mouth cavity (Fig. 4B). This folded lip was comprised of segments I–IV, including eyes 1–3 but not eye 4; and had a "hinge" at IV/V.

Genital region. As with the Lake Phelps leech, the most conspicuous external feature of the Santee leech was a complex copulatory region on the ventral side of genital segments XII and XIII. However, the copulatory zone of the Santee leech differed in displaying a deep U-shaped depression (Fig. 4B). The open ends of the 'U' faced anteriorly, and the bottom of the U was simply defined by annulus XIII a2. The lateral arms of the 'U' were medial to the nephridiopores on each side. The significance of this conspicuous three-dimensional U-shaped feature in this specimen is unknown but it was lacking in specimens from Lake Phelps and Georgia (Fig. 4A, C).

As with the Lake Phelps leech, the male and female openings of the Santee specimen were located at XII b2/a2 and XII b6/XIII b1, respectively, but the genital area differed in minor detail. In the Santee specimen the male opening was a distinctive swollen slit at XII b2/a2. Teasing these two annuli apart revealed a wide, deep opening continuous with the cavity of the male bursa. There was no external evidence of a penis. The female opening was within a deep, mouth-like slit at XII b6/XIII b1. The three annuli between the genital openings, i.e. XII a2–b6, inclusively, were noticeably swollen, obscuring the female opening which ascended behind and over the third annulus. The furrows between the annuli comprising the copulatory region bore distinctive pits, essentially similar to those in the Lake Phelps leech, but with minor variations.

Jaws and teeth. The jaw structure of the Santee specimen was essentially similar to that of the Lake Phelps leech, in that the three jaws were more or less of similar size and shape, with each jaw roughly as long as it is wide. However, in marked contrast to the Lake Phelps leech (Fig. 5A, B) no individually identifiable teeth were detectable in the Santee leech. When the jaws were treated with Haematoxylin (Harris) (Fig. 5C), a thin differentially stained ribbon or plaque ran continuously along the leading edge of the jaw, starting abruptly at the lumenal boundary and tapering off at the distal end where the stain uptake diminished accordingly. This ribbon/plaque clearly represented a monostichodont structure, and there was no evidence for even partial distichodonty.

Even with staining no individual teeth per se were distinguishable. However, the lateral edges of the ribbon/plaque were clearly serrated to reveal the probable number of missing teeth. Within limits of microscopy there were approximately 22–25 teeth on each jaw. This number agreed closely with the 26 individually identifiable teeth reported from a specimen of *P. floridana* collected about 20 miles distance (Richardson 1972). The absence of individual teeth in the Santee leech was attributable to their dissolution in preservative after more than 40 years.

Digestive tract. The digestive tracts of the Santee and Lake Phelps leeches are compared in Fig. 6, with notable differences emphasized as follows. In the Santee leech each respective segment bore two caeca of similar size, the anterior caecum (Fig. 6, acc) facing forward and the posterior caecum (Fig. 6, pcc) facing backward. In contrast, in the Lake Phelps leech the anterior caeca in the mid-body were noticeably larger and more specialized than the posterior caeca.

In the Santee leech the caeca of segments IX–XI were well formed right up to the base of the pharynx. In the Lake Phelps leech the caeca of segments IX–XI were relatively underdeveloped, for the most part bearing rounded protuberances rather than well-formed caeca. The posterior crop caecum of segment XIX (Fig. 6, pcc) bore

irregular expansions along its length, and appeared to be empty, whereas in the Lake Phelps leech this structure was regular and replete with compacted contents.

The first intestinal caecum of the Santee leech was large and well developed behind which were four smaller protuberances at intervals, whereas the intestine of the Lake Phelps leech lacked definitive caeca, apart from the anteriormost. The rectum was relatively small and bore caeca-like protuberances almost as far as the anus; also its lumen bore some rugae. In contrast the rectum of the Lake Phelps leech was conspicuous in size and bladder-like without protuberances; its lumen was smooth.

Gut contents. Unlike the Lake Phelps leech, the entire gut of the Santee specimen was remarkably free of contents. A notable exception was an unidentified species of trematode found in situ in the pouch-like left anterior caecum of the intestine (Fig. 13C, D). This parasite was small (2.5 mm) and remarkably intact, especially considering its unique location. This rounded, slightly curved trematode was leech-like in its shape. The body tapered from a rounded apex at one end to the maximum body width very near the other end of its body. At the latter end a stump-like protuberance projected directly to the posterior. No setae nor spines were evident, but approximately 100 rings were discernible along the circumference of the body. Some remarkably well-preserved internal features were highlighted under transparent lighting. At the narrow end a conspicuous homogeneously dark tube-like structure dominates about half of the available volume. The other end of this worm was occupied by grape-like clumps of a lighter color.

This appears to be the first trematode known from *Philobdella*, and the first record for any leech species in the Carolinas. The interaction of this trematode with its intermediate host, presumably a snail, invites further study. A similar trematode *Alloglossidium macrobdellensis* has been described from the intestine of *Macrobdella ditetra* from Louisiana (Beckerdite & Corkum 1974; Corkum 1975). This trematode is deposited along with its leech host SC(Psa-1) in the Medical Leech Museum.

Nephridia. The nephridia of the Santee leech were unremarkable in size and location (Fig. 6B, N1–17). Specifically, their urinary bladders were inconspicuous, and bore absolutely no physical relationship with the crop caeca. This was in stark contrast to the attachment of huge urinary bladders to the crop caeca in mid-body segments of the Lake Phelps leech (enteronephry) (Figs. 6A, 7).

Reproductive structures. The anatomy of the male and female structures of the Santee leech is illustrated in Fig. 10. In most respects this closely resembled that found in the Lake Phelps leech (Figs. 8, 9), including the presence of gastropores derived from the respective crop caeca of genital segments XII–XIII.

Of possible taxonomic significance discussed later, in the Santee leech the two oviducts independently entered the vagina a short distance behind its distal tip, thus defining a distinct vaginal caecum (Figs. 10, 12A, vc), whereas in the Lake Phelps leech a vaginal caecum was not discernible (Figs. 8, 9). Furthermore, the vertical arm of the vagina was longer than the horizontal arm (Fig. 10, v), whereas in the Lake Phelps leech the vertical arm was noticeably shorter than the horizontal arm (Fig. 8, v).

Description of *P. floridana* from Georgia (Okefenokee Swamp)

A preliminary account of the eco-systematics of *P. floridana* from the Okefenokee Swamp in southeastern Georgia has been presented elsewhere (Hogan & Sawyer 2018). Following is a more comprehensive description of the anatomy of leeches from this population (see Reference material), with particular attention to comparison with *P. floridana* from Lake Phelps, NC. By way of summary, key features of *P. floridana* from Okefenokee Swamp GA(Pfl–2) are illustrated herein (Figs. 3B, D, 4C, 5D, 11, 12B).

Externally, the Okefenokee leech was broadly similar to the Lake Phelps leech NC(Phg–2), bearing in mind that during initial preservation with isopropyl alcohol virtually all pigmentation had dissolved in the former specimen. In agreement with the Lake Phelps leech there were fifteen 5–annulate segments IX–XXIII; and the contiguous annuli VII a3 and VIII a1 were characteristically enlarged, as was annulus XXIV a3. Of possible biological significance the anus was noticeably large (Fig. 3D, arrow), and bordered posteriorly by a fleshy area reminiscent of an annulus, whereas in the Lake Phelps leech the anus was distinctly smaller without evidence of a post-anal prominence.

The copulatory complex resembled that of the Lake Phelps leech, with minor differences. Inexplicably, no mid-ventral slit was evident in the furrow XII b1/b2 anterior to the male gonopore (Fig. 4C; see also Hogan &

Sawyer 2018, fig. 2), whereas the Lake Phelps leech had a prominent slit at this position (Fig. 4A). No clitellum was evident in this specimen, unlike the Lake Phelps leech (Fig. 4A).

Of taxonomic significance the jaws and teeth (Fig. 5D) closely resembled that of the Lake Phelps leech (Fig. 5A, B). Specifically, each jaw was rimmed with 23–26 teeth in a single series (monostichodont). Furthermore, a small pharyngeal ridge terminated independently between each jaw (Macrobdellinae) (Fig. 5D, pr).

The nephridia of the Okefenokee leech were unremarkable, and closely resembled those of the Santee leech (Fig. 6B, N1–17). Most significantly, in marked contrast to the Lake Phelps leech (Figs. 6A, 7), there was no physical association of the nephridial bladders with the crop caeca.

The gut of the Okefenokee leech was essentially similar to that of the Lake Phelps leech in having two crop caeca per segment. However, the caeca in the Okefenokee leech were relatively small and not well developed (Fig. 11A) , in contrast to the Lake Phelps leech in which the caeca were distinct rounded lobes (Fig. 6A).

Another significant difference between the Okefenokee and Lake Phelps specimens was the contents of their guts. That of the Lake Phelps leech was confined to oligochaetes, whereas no oligochaetes were found in the Okefenokee leech. Instead, the latter gut contained what appeared to be torn or cut pieces of invertebrate exoskeleton (Fig. 13B). The presumed scavenging nature of the Okefenokee leech may be a local specialization in that aquatic oligochaetes are very uncommon in the Okefenokee Swamp, and essentially unavailable as a food source (Kratzer & Batzer 2007). The biological significance of this specialized diet is discussed in Hogan and Sawyer (2018).

In most respects the reproductive system of the Okefenokee leech (Fig. 11) closely resembled that of the Lake Phelps leech (Figs. 8, 9). Specifically, the anterior crop caeca of the genital segments XII and XIII were specialized (Fig. 11, acc) and, as tissue integrity allowed, appeared to reach the ventral floor as gastropores. Both leeches had seven pairs of testisacs. However, the seventh or last testisac of the Okefenokee leech lacked the integrity of the first six and could easily be overlooked (Fig. 11A, T1–T7). The female system agreed with the Lake Phelps leech in lacking a common oviduct (Fig. 11), as well as lacking a vaginal caecum (Fig. 12B) so clearly present in the Santee leech (Fig.12A, vc). Similarly, the relative lengths of the two arms of the vagina agreed with the Lake Phelps leech (Figs. 8, 11B, v), but differed from the Santee specimen in which the vertical arm was distinctly longer than the horizontal (Fig. 10A, v).

Taxonomic discussion

Mouthparts and feeding biology. All specimens examined in this study possessed remarkably similar jaws and teeth within the range of 22–26 per jaw, regardless of the size or origin of the leech (Fig. 5). This conforms to the original description of *P. floridana* which had "about twenty acute teeth" (Verrill 1874), and is compatible with diagnosis of this species prior to this study (Phillips & Siddall 2005; Moser *et al.* 2011).

All evidence in this paper and elsewhere (Hogan & Sawyer 2018) indicates that *P. floridana* is a macrophagous predator and opportunistic scavenger which feeds primarily on aquatic oligochaetes (Fig. 13A, B). This is consistent with the feeding behavior of the holotype from Lake Okeechobee, FL, which "was engaged in swallowing a small lumbricoid worm" (Verrill 1874). Counterintuitively for a jawed monostichodont leech, there is no record in the Carolinas, Georgia or Florida of *P. floridana* sucking the blood of any vertebrate, including humans.

Furthermore, as shown for the first time in this study (Figs. 6A,B; 11A), the gut of *P. floridana* is distinctly macrophagous. It is compartmentalized by segmental, intra-lumenal sphincters and lined with prominent rugae, perhaps of a grinding function. The rugae were especially pronounced toward the posterior end of the midgut.

The mouthparts of *P. floridana* differ significantly from those of the only other member of the genus, *P. gracilis* Moore, 1901, of the Mississippi drainage system. The latter species has 35–45 "partially distichodont" teeth per jaw (Moore 1901a; Klemm 1982; 1985; Phillips & Siddall 2005; Moser *et al.* 2011). This raises the biological question whether the two species of *Philobdella* differ also in their feeding habits, as one might expect. On the one hand Moore (1901) confirmed the remains of aquatic oligochaetes in the gut of *P. gracilis.* On the other hand, Viosca (1962) claimed this species can be sanguivorous on aquatic amphibians and reptiles. However, the feeding biology of *P. gracilis* must be critically revisited in that Viosca did not distinguish between being attached to a host and feeding on it. Furthermore, his brief note was published posthumously forty years after it was written

in 1922. Unfortunately, nothing is known of the internal anatomy of *P. gracilis* since Moore's incomplete account more than a century ago.

Copulatory complex and gastropores. This study is the first demonstration of gastropores in any leech species native to North America. This intricate network arising from specialized crop caeca of the genital segments XII and XIII constitutes the anatomical framework underpinning the external copulatory pits and slits which make the genus *Philobdella* uniquely recognizable (Figs. 4, 8–11). In this context, it is established within the Hirudinea generally that a hole or pore is formed when crop endoderm comes into contact with ectoderm, e.g. formation of the anus (Sawyer 1986: 513–514). In other words, gastropores are in essence secondary anuses.

The function of gastropores in the biology of *P. floridana* is conjectural, but a role in reproduction is a strong possibility. Notably, gastropores were absent in the immature specimen from Lake Phelps (NCSM 47412). In adults of this species, the gastropores are invariably in close proximity to both male and female gonopores. However, the female system is far more intimately involved. In fact, there is internal evidence they may form a symmetrical ring around the female orifice. Speculatively, this intricate network may serve some kind of hydraulic and/or moistening function during mating and/or cocoon deposition. This correlates with the 'primitive' nature of the *Philobdella* reproductive anatomy, i.e. absence of a true penis, as well as a common oviduct (Figs. 8–11).

Vaginal caecum. Richardson (1972) reported the presence of a vaginal caecum in *P. floridana* from the Santee Swamp of coastal South Carolina. Being the only dissection of this species prior to this study, it had been assumed until now that a caecate vagina was characteristic of the genus *Philobdella* (e.g. Sawyer 1986: 683, 686). The taxonomic significance is that this feature is not known in any other jawed leech from North America, including other Macrobdellinae (see Sawyer 1986: 683–684, 688).

In seeming corroboration, this study confirmed the unequivocal presence of a vaginal caecum in another specimen of *P. floridana* from a nearby locality in South Carolina (Fig. 12A). However, specimens of *P. floridana* from North Carolina (Lake Phelps) and Georgia (Okefenokee Swamp) did not possess a caecate vagina (Fig. 12B). Accordingly, such a feature may be confined to *P. floridana* from coastal South Carolina, and in any case can no longer be a characteristic of the genus *Philobdella*.

Taxonomic revision

The foregoing descriptions of *Philobdella floridana* from North Carolina, South Carolina and Georgia revealed that these widely disparate populations shared a number of external and internal features of taxonomic significance. Taken together these key characters can serve as the basis for a taxonomic revision of *P. floridana*, the type species for the genus, summarized succinctly as follows:

Philobdella floridana (Verrill, 1874), Revised

Partial Synonymy:
Macrobdella (Philobdella) floridana Verrill, 1874.
Philobdella gracilis: Richardson (1972); Sawyer & Shelley (1976); Sawyer (1986).
Philobdella floridana: Phillips & Siddall (2005); Moser *et al.* (2011); Hogan & Sawyer (2018).

Diagnosis: Eyes 5 pairs in an arc (Hirudinidae); a pharyngeal ridge terminating independently between each jaw (Macrobdellinae); adults with a complex copulatory region characterised by pores and slits (*Philobdella*); jaws armed with 20–26 monostichodont teeth (*P. floridana*); male and female gonopores separated by three annuli, located at XII b2/a2 and XII b6/XIII b1, respectively;.

Description: A continuous mid-dorsal light stripe, on either side of which are three dark stripes of varying integrity; darkish dorsum sharply demarcated at margins from an unpigmented venter (apart from a few scattered ventral blotches). 15 complete (5-annulate) segments, IX–XXIII, inclusively; segment V forms posterior rim of oral sucker; segment VII, 3-annulate; VIII, 4-annulate; the two contiguous annuli VII a3 and VIII a1 characteristically enlarged; segment XXIV, 4-annulate, with XXIV a3 characteristically enlarged; segment XXV, 4-annulate; crop with two pairs crop caeca per segment, designated anterior and posterior (Fig. 7); anterior caeca of segments XII and XIII penetrate to ventral body wall of the copulatory region (gastropores); midgut

compartmentalized by segmental, intra-lumenal sphincters; 7 pairs of testisacs, in segments XIV–XX, inclusively; no true penis, and no common ejaculatory ducts nor ejaculatory bulbs; vagina present, but no common oviduct.

Conclusion

A key question posed in this study is the taxonomic status of a newly discovered population of *P. floridana* from a remote lake in northeastern North Carolina, by far the northernmost record for this species. Description of the Lake Phelps leech in this paper is the first comprehensive account of the internal anatomy of *P. floridana* (Figs. 6A, 8, 9). Comparable internal descriptions of *P. floridana* from other geographic regions, namely South Carolina (Figs. 6B, 10) and Georgia (Fig. 11), are also presented for the first time. This new understanding of the internal anatomy of this species is the basis of a taxonomic revision of *P. floridana*.

A major finding is that the Lake Phelps population differs significantly from other known *P. floridana* in one significant respect. Specifically, the gut has an intimate anatomical association with the nephridia (Figs. 6A, 7), an enteronephric feature previously unknown within the Hirudinea (Sawyer 1986: 113–132). From the foregoing, at least in this study, enteronephry in this species may be confined to Lake Phelps, leaving open the possibility that this population of *P. floridana* may indeed be endemic to this relict lake which has a history of endemicity (see Introduction).

The biological significance of an association between the gut and nephridia in the Lake Phelps population is speculative. However, an analogous phenomenon is known among certain terrestrial Oligochaeta. This was first reported by Bahl (1919) who showed that segmental nephridia of the earthworm *Pheretima* open directly into the gut, for which he coined the term 'enteronephric'. Subsequent biological studies of this and related oligochaete genera concluded this enteronephry is a mechanism for water conservation (Little 1983: 25). In support of this view, in its more than 300 years of recorded history, Lake Phelps has experienced great droughts, sometimes for periods of more than 18 months, during which the water level fell more than four feet (Sawyer 2010: 171). In other words, enteronephry in the Lake Phelps population may be viewed best as manifestation of local adaptation, without wider taxonomic implications.

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References

Anonymous (1979) *Lake Phelps. Lake Management Study*. North Carolina Department of Natural Resources, Division of Parks and Recreation, Raleigh, NC, 196 pp.

Bahl, K.N. (1919) On a new type of nephridia found in Indian earthworms of the genus *Pheretima*. *Journal of Cell Science*, 2–64, 67–117.

Beckerdite, F.W. & Corkum, K.C. (1974) *Alloglossidium macrobdellensis* sp. n. (Trematoda: Macrobderoididae) from the leech, *Macrobdella ditetra* Moore, 1953. *Journal of Parasitology*, 60, 434–436.
https://doi.org/10.2307/3278357

Corkum, K.C. (1975) Observations on the life history of *Alloglossidium macrobdellensis* (Trematoda: Macrobderoididae) from *Macrobdella ditetra* (Hirudinea: Hirudinidae). *American Midland Naturalist*, 93, 484–491.
https://doi.org/10.2307/2424186

Hogan, J.I. & Sawyer, R.T. (2018) The leech *Philobdella floridana* (Verrill, 1874) (Annelida: Hirudinea) reported from the Okefenokee Swamp, Georgia, USA, with observations on its biology. *Florida Scientist*, 81, 70–79.

Klemm, D.J. (1982) *Leeches (Annelida: Hirudinea) of North America.* United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 177 pp.

Klemm, D.J. (1985) Freshwater leeches (Annelida: Hirudinea). *In*: Klemm, D.J. (Ed), *A Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America.* Kendal/Hunt Publishing Company, Dubuque, IA, pp. 70–198. [not seen]

Krabbenhoft, T.J., Rohde, F.C. & Quattro, J.M. (2008) Threatened fishes of the world: *Fundulus waccamensis* (Hubbs and Raney, 1946) (Fundulidae). *Environmental Biology of Fishes*, 84 (2), 173–174.
https://doi.org/10.1007/s10641-008-9401-3

Kratzer, E.B. & Batzer, D.P. (2007) Spatial and temporal variation in aquatic macroinvertebrates in the Okefenokee Swamp, Georgia, USA. *Wetlands*, 27, 127–140.
https://doi.org/10.1672/0277-5212(2007)27[127:SATVIA]2.0.CO;2

Little, C. (1983) *The Colonisation of Land: Origins and Adaptations of Terrestrial Animals.* Cambridge University Press, Cambridge, 290 pp.

Moore, J.P. (1901a) The Hirudinea of Illinois. *Bulletin of the Illinois State Laboratory*, 5, 479–547.

Moore, J.P. (1901b) Descriptions of two new leeches from Porto Rico. *Bulletin of the United States Fish Commission*, 2, 211–222.
https://doi.org/10.5962/bhl.title.58580

Moser, W.E., Klemm, D.J., Phillips, A.J., Trauth, S.E., Neal, R.G., Stanley, J.W., Connor, M.B. & Flotemersch, J.E. (2011) Distribution of the genus *Philobdella* (Macrobdellidae: Hirudinida), including new locality records from Arkansas and Oklahoma. *Comparative Parasitol*ogy, 78, 387–391.
https://doi.org/10.1654/4507.1

Owen, D.A. (2016) *Historic Data 1981–2015, Lake Phelps*. North Carolina Division of Water Resources, NC Department of Environmental Quality, Raleigh, NC.

Phillips, A. & Siddall, M. (2005) Phylogeny of the new world medicinal leech family Macrobdellidae (Oligochaeta: Hirudinida: Arhynchobdellida). *Zoologica Scripta*, 34, 559–564.
https://doi.org/10.1111/j.1463-6409.2005.00210.x

Richardson, L.R. (1972) On the morphology and nature of a leech of the genus *Philobdella* (Hirudinoidea: Macrobdellidae). *American Midland Naturalist*, 87, 423–433.
https://doi.org/10.2307/2423573

Sawyer, R.T. (1986) *Leech Biology and Behaviour*. Oxford University Press, Oxford, 1065 pp.

Sawyer, R.T. (2010) *America's Wetland. An Environmental and Cultural History of Tidewater Virginia and North Carolina*. University of Virginia Press, Charlottesville, VA, 260 pp.

Sawyer, R.T. & Shelley, R.M. (1976) New records and species of leeches (Annelida: Hirudinea) from North and South Carolina. *Journal of Natural History*, 10, 65–97.
https://doi.org/10.1080/00222937600770061

Speiran, G., Wurster, F. & Eggleston, J. (2015) The groundwater geochemistry of the Great Dismal Swamp, a peat wetland discharging to two mid-Atlantic estuaries, USA. *Geological Society of America, 2015 GSA Annual Meeting*, Baltimore, MD, 1–4 November 2015. Available from: https://gsa.confex.com/gsa/2015AM/webprogram/Paper267381.html (accessed 11 December 2018)

Stuber, D. & Scanlan, J. (2016) Some observations on the Waccamaw Killifish (*Fundulus waccamensis)* with notes on spawning in captivity. *American Currents*, 41, 19–23.

Verrill, A.E. (1874) Synopsis of North American freshwater leeches. *United States Fisheries Commission Report for 1872–73*, Pt. 2, 666–689.

Viosca, P. (1962) Observations on the biology of the leech *Philobdella gracile* Moore in southeastern Louisiana. *Tulane Studies in Zoology*, 9, 243–244.

ABBREVIATIONS TO FIGURES. The following abbreviations and shading conventions are used in figures throughout this paper: acc, anterior crop caecum; c, crop; cm or cp, circumpharyngeal commissure; e, epididymis; ed, ejaculatory duct; g, ganglion; i, intestine; j, jaw; m or mb, male bursa; N, urinary bladder; o, ovisac; od, oviduct; p, pharynx; pcc, posterior crop caecum; r, rectum; T, testisac; T1, anteriormost or 1st testisac; T7, posteriormost or 7th testisac (underdeveloped); v, vagina; vc, vaginal caecum; vd, vas deferens; ve, vas efferens; vnc, ventral nerve cord. Stippled, crop and its caeca; shaded, urinary bladders (N1–N17); solid spheres, segmental ganglia (I–XXXIV); Roman numerals refer to respective body segments.